1 *Review*

2 **A review of the impact of xenobiotics from dietary sources on infant health: early life**
3 **exposures and the role of the microbiota**

4
5
6 Calatayud Arroyo M.^{1*}, García Barrera T.², Callejón Leblic B.², Arias Borrego A.², Collado
7 M.^{1*}

8
9 1 Department of Biotechnology, Institute of Agrochemistry and Food Technology, Spanish
10 National Research Council (IATA-CSIC), Valencia, Spain

11 2 Research Center on Natural Resources, Health and The Environment (RENSMA).
12 Department of Chemistry “Prof. J.C. Vilchez Martín”. University of Huelva. Fuerzas
13 Armadas Ave., 21120 Huelva (Spain).

14
15 * Corresponding author: Marta Calatayud Arroyo; Department of Biotechnology, Institute of
16 Agrochemistry and Food Technology, Spanish National Research Council (IATA-CSIC),
17 Valencia, Spain; catalasa@iata.csic.es

18 Maria Carmen Collado; Department of Biotechnology, Institute of Agrochemistry and Food
19 Technology, Spanish National Research Council (IATA-CSIC), Valencia, Spain;
20 mcolam@iata.csic.es

21
22 **Keywords:** microbiota, diet, infant, xenobiotic, breastfeeding
23

Abstract

Xenobiotics are worldwide distributed and humans are unavoidably exposed to multiple chemical compounds during life, from preconception to adulthood. The human microbiota is mainly settled during early life and modulate host health and fitness. One of the main routes for chemical exposure is by intake of contaminated food and water. Thus, the interplay between diet-xenobiotics-microbiota during pregnancy and perinatal period may have relevant consequences for infant and adult health. Maternal exposure to metal(oid)s, persistent organic pollutants, and some food additives can modify the infant's microbiota with unknown consequences for child or adult health. Toxicants' exposure may also modulate the maternal transfer of microorganisms to the progeny during birth and breastfeeding; however, scarce information is available. The rapid increase in releasing novel chemicals to the environment, the exposure to chemical mixtures, the chronic/low dose scenario, and the delay in science-stakeholders action call for novel and groundbreaking approaches to improve a comprehensive risk assessment in sensitive population groups like pregnant women and neonates, with emphasis on microbiota as modulating factor and target-organ of xenobiotic's toxicity.

Capsule

Parental and subsequent lifetime chemical exposures interact with the developing human microbiota at critical windows of development, likely affecting health outcomes in infancy and adult life.

Introduction

The human body establishes a dynamic symbiosis with bacteria, viruses, fungi, archaea, and bacteriophage communities from the beginning of life (Milani et al., 2017). The establishment of the human holobiont, encompassing from fetal development to adulthood, is influenced by the interplay among complex microbial communities, the host and the environment (Simon et al., 2019). Together with individual factors, environmental parameters related to diet, indoor and outdoor exposures shape the human microbiome (Adams et al., 2015; Christoff et al., 2019). The exposome's effect on human health and the bidirectional interplay between chemicals' exposure and human microbiota is currently recognized as a critical factor shaping human health (Mapesa et al., 2016; Renz et al., 2017).

The first 1000 days of life (from conception to two years) supposes a critical window of childhood growth and development, characterized by the maturation and epigenetic programming of metabolic, endocrine, neural and immune pathways (Robertson et al., 2019). These fast developmental processes are accompanied by the establishment of the human microbiome, which may modulate human development. The Developmental Origins of Health and Disease or Barker hypothesis established causality between adverse effects occurring *in utero* (e.g., environmental pollutants, toxic metal(loid)s) and the etiology of diseases in adulthood (Heindel, 2020; Heindel et al., 2015).

Foetus, neonates, infants and children are especially sensitive to toxicants due to the relative immaturity in metabolizing enzymes and lower capacity to eliminate toxic compounds (toxicokinetics) or due to the high sensitivity of target organs (toxicodynamics) (Simon et al., 2019). The severity of xenobiotic exposure during infancy is correlated to the existence of windows of vulnerability or developmental windows when cellular functions as maturation,

differentiation, growth, and epigenetic programming are susceptible, and the possible effects may be permanent.

Exposure to inorganic and organic chemicals, including endocrine-disrupting agents and metal(loid)s are a global worldwide concern (Goodrich et al., 2019). In the general population, the primary source of exposure to pesticides and heavy metals is through diet. A wide array of chemicals have been described in different food matrices as vegetables, fish, meat, eggs, and cereals and thus, maternal consumption of contaminated foodstuff can end up with foetal exposure to a multitude of chemicals affecting all organ systems (nervous, cardiovascular, respiratory, reproductive and immune).

Transfer of toxic chemicals from maternal to foetal compartment across the placenta is analogous to the transport processes across other biological membranes, increasing the rate exchange as the foetal growth rate increases (V. E. Murphy et al., 2006; Porpora et al., 2013). After birth, oral, dermal, and respiratory routes also account for the neonate's exposure to multiple chemicals.

Remarkably, our current safety assessment and knowledge on the toxicant's effect and mode of action is primarily based on acute, single-dose, and single-substance exposure in adult populations. Still, the potential risk posed by simultaneous contact with hundreds of chemicals at low doses and in the long-term scale is still to be determined (Box 2), primarily when exposure occurs during prenatal and early life periods (Renz et al., 2017).

The worldwide increase on the prevalence of non-communicable diseases, such as cardiovascular disease, diabetes, obesity, and cancer, may be influenced by exposure to different stressors during foetal or postnatal development, a hypothesis postulated several years ago as the Developmental origins of health and disease (DOHaD) (Barker, 2007). More recently, toxicomicrobiomics studies have integrated the environmental and human microbiota to toxicology studies, generating relevant evidence of the mutual and dynamic

influence between the microbiome cloud and xenobiotics of various origins (Abdelsalam et al., 2020). However, toxicomicrobiomics in neonatal and infant populations is still underrepresented in research.

In this review, we summarized the current evidence on the interplay between maternal-infant dyad microbiota and xenobiotics, with a primary focus on the effect of xenobiotics from dietary sources on maternal and infant microbiota.

Methodology

This scoping review aims to survey the available evidence on the interplay between xenobiotics and maternal-neonatal microbiota, including chemical substances present in food (e.g., organic and inorganic chemicals, food industry ingredients such as sweeteners or emulsifiers) and the possible effects on infant health.

A literature search was conducted in Scopus, Web of Knowledge, and the Cochrane Library. Terms used in the analysis were the following: “breastmilk or infant or neonate or fetus or prenatal” and “microbiota or microbiome” and “toxicant(s) or environmental pollutant(s) or chemical(s) or xenobiotic(s) or heavy metal(s) or exposome or sweeteners or emulsifiers or microplastics or nanoparticles”, included in the topic, abstract, title or keywords. References to antibiotics or antimicrobials were not included in the review, as we consider it is a broad topic that would require specific attention. The selection of the different groups of xenobiotics included in the research was based on previous description in food items.

When less than ten publications were found for a combination of terms, a Google Scholar search was conducted, including different combinations of words in the article’s full length. When the literature search did not deliver any result, “microbiota or microbiome” or “breastmilk or infant or neonate or fetus or prenatal” was eliminated, and identification of

relevant studies that could relate microbiota with health effects was made through hand search in original articles on the subject. Results reporting relevant information on microbiota and toxicants interplay in human adult populations and/or animal studies were only included when no information or limited references in infants/neonates were found.

Inclusion criteria covered human epidemiological studies reporting population-based cohort, cross-sectional or case-control studies, and animal (mammal) studies assessing xenobiotics' exposure quantitatively. No minimum number of participants in the studies was applied. Documents should be original and had to be peer-reviewed and published in English. Research and review articles, meeting abstracts, books, and other types of publications (e.g., preprints) were considered. The literature search was performed until September 2020 and had no limit in years. Review articles were filtered from the search and only used to support the findings, but not as a primary source, otherwise stated in the text.

Human-made chemical substances unavoidably surround us.

Food contamination by chemicals is a major global food safety issue, arising a serious threat to human health. The source of food contamination can be anthropogenic or naturally-derived from the environment. At the same time, multiple groups of chemicals have been described in food items, belonging to metal(oid)s, polycyclic aromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), perfluorinated compounds (PFCs), radioactivity, plastics, and nanoparticles (reviewed in (Heindel et al., 2015; Thompson & Darwish, 2019)). Besides, some ingredients used in food processing can also have undesirable consequences on consumer's health when consumed in high doses, such as artificial sweeteners or emulsifiers (reviewed in (Abdelsalam et al., 2020)). For most human and animal populations, the intake of contaminated food is the primary source of exposure (González et al., 2019). In addition to the compounds currently found in the environment, new chemicals are produced and the

environmental pool of xenobiotics is alarmingly increasing. In 2020, an updated version of the Toxic Substances Control Act (TSCA) Inventory by the U.S. Environmental Protection Agency (EPA) reported around 86.000 chemicals available for commercial production and use in the U.S. (EPA, 2020). The U.S. legal environment permits post-marketing evaluation of health or environmental risks (Grandjean et al., 2019), thus allowing the production, use, and distribution of chemical compounds without previous risk assessment. The EU REACH legislation requires certain toxicological information before chemicals marketing. However, the multi- and transgenerational effects and the prenatal vulnerability are usually overlooked in risk estimation (Kostoff et al., 2018; Aristidis Tsatsakis et al., 2019). The steady increase in the volume of synthetic chemicals produced worldwide (CEFIC, 2020) overwhelms the system's capacity for accurate testing of hazardous compounds. Specifically, adverse effects of toxicants may result from exposure before conception (paternal and/or maternal) (Bailey et al., 2020; Mustieles et al., 2020), prenatal development, or postnatal to the time of full maturity. Thus, toxic imprinting in early-life and thereafter during adulthood is a current scenario that needs to be considered for environmental hazard protection.

Findings

Mother-infant dyads and their microbiota, the unexplored interplay with xenobiotics

Despite the prenatal exposome is starting to be evaluated (reviewed in Rager et al., 2020) (Rager et al., 2020), little is known about the interaction of environmental chemicals and the human microbiome at different anatomical locations, especially during pregnancy and early life.

Most of the human microbiota is acquired from maternal-infant transmission at birth and during breastfeeding, followed by environmental acquisition at later stages (Mueller et al., 2015; Wang et al., 2020). Findings of bacterial DNA in the placenta or amniotic fluid

(Aagaard et al., 2014; Chu et al., 2017; Collado et al., 2016), or the presence of bacteria-like structures in fetal meconium (Rackaityte et al., 2020) have challenged the well-established paradigm of the fetal-sterile environment, however, the majority of the human microbiota seems to be acquired after birth (Lauder et al., 2016; Theis et al., 2019). Even if only pathogens can be detected into pre-term or complicated pregnancies (B. Cao et al., 2014; de Goffau et al., 2019), or in the case of a true placental/amniotic microbiota, the relevance of the interaction between xenobiotics and certain pioneer microorganisms or bacterial DNA on fetal immune priming will require further characterization.

Sensitive periods of life for metabolic, immune, and microbial programming (e.g., pregnancy, prenatal development) are usually overlooked in toxicants' risk assessment. Gestation is associated with many physiological changes that can influence the uptake, absorption, distribution, metabolism, and excretion of xenobiotics in both the mother and in the developing fetus. For example, decreased intestinal motility and longer gastric emptying times during gestation can increase the absorption of certain chemicals (Organization, 2006). Pregnancy and post-partum have also been associated with significant changes in gut microbiota, metabolic syndrome, fat redistribution, reduction of plasma albumin levels, increased epithelial permeability and plasma endotoxin levels (Kerr et al., 2015). These factors can affect the distribution and possible effect of xenobiotics in the mother-infant dyad.

In 2002, Baillie-Hamilton proposed a link between rising obesity rates worldwide and environmental pollutants (Baillie-Hamilton, 2002). Since then, the "obesogens hypothesis" has been demonstrated in human, animal, and *in vitro* studies, linking environmental chemicals (e.g., dichlorodiphenyldichloroethylene, hexachlorobenzene, polychlorinated biphenyls (PCBs), dioxins, some insecticides, and herbicides) and multiple molecular targets (estrogen receptor, retinoid X receptor, peroxisome proliferator-activated receptor-g, and

glucocorticoid receptors) involved in adipogenesis, lipid metabolism, or energy balance (Heindel, 2020).

Gut microbiota is linked with the host's health status, affecting metabolic, endocrine, and neurodevelopmental paths (Dominguez-Bello et al., 2019), yet little is known on the bidirectional interplay of the microbiota and environmental chemicals in mother-infant dyads. Thus it is time to incorporate human microbiota in updated paradigms of toxicology.

In that sense, some transient microorganisms can pave the way for more stable resident microbiota (Sprockett et al., 2018), while the effect of breastfeeding habits and transition to solid food significantly contribute on infant gut microbiota development and configuration (Bäckhed et al., 2015; Timmerman et al., 2017). Other body sites, including the oral cavity, lungs, and skin, are also widely populated by commensal microorganisms. At the same time, acquisition and patterns are also dependent on maternal vertical transmission and environmental exposures. Therefore, the maternal influence on microbiota during the prenatal and early postnatal period is essential. The “filtering” effect of the mother in terms of exposome transmission to the fetus and newborn is critical but poorly understood, and we termed “the exposome legacy”. Maternal exposure to environmental chemicals is therefore transferred via umbilical cord circulation, placenta, or breastfeeding, however, metabolic changes on different compounds can be expected by maternal biotransformation, mainly occurring in the liver or by the gut microbiome (Figure 1). Various xenobiotics interplay with the human microbiota in a two-way interaction entailing the microbial biotransformation of chemicals (Koppel et al., 2017) and, on the other hand, the modulation of the microbiota by specific chemicals or mixtures (Dietert & Silbergeld, 2015; Jin et al., 2017; Sbihi et al., 2019). In that sense, the gut microbiota has a broad repertoire of enzymes not present in the host, leading to the inactivation of active compounds, bioactivation of prodrugs, or production of newly toxic metabolites (S. L. Collins & Patterson, 2020). On the other hand,

xenobiotics affect the gut microbiome in unpredictable ways, influencing the development of metabolic diseases (reviewed in (Tsiaoussis et al., 2019)), but most of our knowledge is based on human or animal studies in adult populations.

Evidence on the interplay between metal(-oid)s and infant microbiota

Human studies

Rothenberg et al., 2019 reported associations between mercury (Hg) biomarkers in maternal stool, blood and hair, and also meconium, and intestinal taxa, especially during early gestation (n = 52) (Table 1). Inorganic Hg in stool was positively correlated with *Prevotella* relative abundance, while blood and meconium methylmercury (MeHg) were negatively correlated with Ruminococcaceae family members. Authors concluded that associations between gut microbiota and MeHg biomarkers might influence MeHg exposure (Rothenberg et al., 2019), however, further confirmation is required. Previously, the same authors reported that differences in MeHg biomarkers in pregnant women were not likely caused to direct gut microbiota metabolism, but suggested indirect pathways (e.g., modulation of intestinal permeability) linking gut microbiota and Hg intestinal exposure (Rothenberg et al., 2016). Butyrogenic bacteria belonging to the Lachnospiraceae and Ruminococcaceae families have been considered a marker of intestinal health, while their depletion is correlated with reduced production of SCFAs and pathological intestinal inflammatory conditions (Milani et al., 2017). Despite the relevance of the results, the study's limited sample size (n = 7) would require further investigation.

In a study of 179 six-week-old infants, early postnatal toxic and nutrient elemental exposures are associated with modifications in the infant microbiome (Laue et al., 2020). Lower diversity was found in infants exposed to higher levels of arsenic, compared to the full population. Effects of arsenic on microbial diversity were enhanced in infants exposed to

peripartum antibiotics. Most of the analysed toxic and essential elements (arsenic, cadmium, copper, iron, lead, manganese, nickel, selenium, tin, and zinc) were associated with changes in at least one taxon, especially those belonging to *Bacteroides* and Lactobacillales. *Bifidobacterium* was negatively associated with zinc concentrations and had quadratic associations with arsenic, with overall changes influenced by other elements' concentration. In a group of US infant population (n = 204), low levels of urinary arsenic were related to gut microbiome composition at six weeks of life. Among other genera, *Bacteroides* and *Bifidobacterium* are negatively associated with urinary arsenic, in a sex-dependent manner (Hoen et al., 2018).

Remarkably, bifidobacterial abundance has been associated with positive health status. Decreased levels of *Bifidobacteria* spp. in the infant's intestine are correlated with the development of obesity, celiac disease, allergy, and some autoimmune diseases later in life (Milani et al., 2017). The *Bifidobacterium* and *Bacteroides* genera are the major groups of human milk oligosaccharide (HMO)-metabolizing bacteria, promoting a suitable environment for early-life microbial engraftment (Ogra et al., 2020). In infants exposed to antibiotics, depletion of *Bifidobacteria* and *Bacteroides* spp. can promote enterobacteria and enterococci overgrowth, thus deregulating ecological homeostasis and inducing dysbiotic patterns (Milani et al., 2017). Certain *Bifidobacteria* spp. can modulate the intestinal barrier function and *Bifidobacterium* spp. and *Bacteroides* spp. seem to have anti-inflammatory properties, likely mediated by TLR9 enhancement of epithelial integrity and by influencing immune maturation (Milani et al., 2017; Vatanen et al., 2016). Furthermore, some *Bacteroides* spp. produce polysaccharide A with tolerogenic activity through induction of regulatory T cells in mice; however, specific *Bacteroides dorei* lipopolysaccharide may prevent early education of the mucosal immune system and contribute to alterations in immune maturation leading to autoimmune conditions (Vatanen et al., 2016).

Thus, metal(oid)s induced dysbiosis can likely promote alterations in microbial colonization, intestinal homeostasis (e.g., intestinal permeability, low-grade inflammation), and immune priming, consequently impairing lifelong health.

Animal studies

Lead (Pb) shaped gut microbiome of a mouse model after perinatal exposure to 32 mg/L for 40 weeks, causing a decrease in the Bacteroides/Firmicutes ratio. Despite significant changes in the gut microbiota's function and activity, only bodyweight increase was observed in females, pointing out the possibility of sex-specific responses to the chemicals exposure (Wu et al., 2016).

Changes in Firmicutes/Bacteroidetes ratio have been linked to childhood obesity (Indiani et al., 2018), but accumulating evidence from human and animal studies also showed that the relative abundance of the Firmicutes and Bacteroidetes phyla is highly variable, and contradictory results were observed when comparing the microbiota between normal-weight and obese groups (Magne et al., 2020; E. F. Murphy et al., 2010) . Incorporating lifestyle factors (e.g., diet, physical activity) and xenobiotics' exposure can help to understand changes in major gut components.

In a rat model, exposure of pups (5d) to copper (0, 0.04-1mg/kg BW) caused a systemic inflammatory response, increased intestinal permeability, and gut microbiota shifts. Concretely, the abundance of *Treponema* and *Erysipelatoclostridium* had a positive association with copper exposure concentration, while the abundance of *Romboutsia*, *Chlamydia*, *Bifidobacterium*, and *Lactobacillus* decreased with increasing copper exposure concentration (Dai et al., 2020). In humans, a reduction of lactobacilli and bifidobacteria has been associated with immune disorders as atopic disease, inflammatory bowel disease or asthmatic phenotype (reviewed in (Tojo, 2014)).

In a mice model, six weeks of arsenic exposure (50 and 500 ppb) and zinc restriction affected host physiology and gut microbiota. The abundance of *Akkermansia* and *Clostridium* was positively associated with arsenic concentration, while *Ruminococcus*, unclassified *Clostridiales*, *Lachnospiraceae*, and *Erysipelotrichaceae*, among others, were negatively associated with arsenic exposure (Gaulke et al., 2018). Authors demonstrated that zinc deficiency sensitized the gut microbiota to arsenic exposure, pointing out the relevance of accounting for the interactions between diet (e.g., essential nutrients) and environmental toxicants exposure when reporting gut microbiota changes. Species from *Akkermansia* and *Ruminococcus* genus are associated with a health condition and described as intestinal-mucus degraders. Arsenic induces changes in gut microbiota associated with mucus in human *in vitro* models (Calatayud Arroyo, M., Ossieur, W., & Van de Wiele, T., 2016), and it is known that interact with thiol groups, present in mucus surfaces in cysteine residues. The consequences of mucus-dysbiosis on host health will require further research.

Evidence on the interplay between organic chemicals and infant

Human studies

Phthalate metabolites and concretely diethylhexyl phthalate have been reported to alter infant gut microbiota composition and diversity (n = 25), significantly decreasing *Rothia* spp. and *Bifidobacterium longum* (Yang et al., 2019). On the other hand, phthalates' bacterial metabolism and binding abilities have been reported (Yuan-ting Zhu et al., 2018), likely connecting the interplay between phthalate exposure, metabolism, and gut microbiota with phthalate toxicity. *Rothia* has main functions gastrointestinal tract, with a high capacity to ferment various carbohydrates and amino acids and produce enterobactin (Uranga et al., 2020), while *B. longum* (subsp. *longum*) has been described ubiquitously across the human lifespan acquired mainly through mother-infant transfer and interaction with relatives. Not

only the consequences for the infant's health, but also for the transgenerational transmission of the microbiota should be considered.

Laue et al., 2019 showed associations between polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) exposure during pregnancy and gut microbiota of infant aged 6-8 years old (n = 18). Higher exposure to PCB (PCB153, PCB180) specific congeners during pregnancy was associated with a higher relative abundance of Propionibacteriaceae in mid-childhood, while higher PCB-180 and Σ 3PCB were associated with a higher relative abundance of Bacillales Family XI. Higher PBDE-99 exposure was associated with a decrease in uncultured bacteria within the Ruminococcaceae NK4A214 group, and PBDE-47 was related to differences in *Ruminococcus* genus (Laue et al., 2019). This study was conducted in a homogeneous population of women “*who ever breastfed the child subject*”, however, no data reporting exclusive or mixed breastfeeding and duration of breastfeeding was reported, a factor likely affecting gut microbiota composition.

Thus, *in utero* exposure to environmental chemicals may shift the childhood microbiome's composition, affecting functional microbiota abilities that may mediate adverse effects during infancy and adulthood. Propionibacteria produce short-chain fatty acids, cyanocobalamin, and other useful compounds for their hosts (J. K. Collins et al., 1998), and members of the genus *Propionibacterium* have been identified in infant feces at an early age, probably acquired from maternal skin (Ferretti et al., 2018). It has also been described that cesarean section (CS) influences infant's microbiome, which has microbiota similar to the skin including *Staphylococcus*, *Corynebacterium* and *Propionibacterium* as the dominant species (Zhuang et al., 2019). *Propionibacterium* has been defined as proteolytic and “unhealthy” proinflammatory bacteria (Askarova et al., 2020). However, the enrichment of *Propionibacterium* species in breastfed infants has been shown to induce immunoregulation, which safeguards against proinflammatory diseases (Colliou et al., 2017). On the other hand,

Ruminococcaceae families encompass highly diverse species, many of which produce beneficial SCFAs that influence intestinal health (Milani et al., 2017).

Another study reported the impact of a 28 chemical exposures, including polychlorinated biphenyls (PCBs), polybrominated flame retardants (PBDEs), per- and polyfluoroalkyl substances (PFASs), and organochlorine pesticides, present in breast milk in the neonatal gut microbiota (Iszatt et al., 2019) (n=239). Infants in the high exposure group had a lower abundance of different *Lactobacillus* genus than the low exposure group. *Lactobacillus* spp. are considered probiotic microorganisms with beneficial effects on immune regulation and intestinal barrier and had been described in higher levels in breastfed infants (Gao et al., 2019; Gaucher et al., 2019; Wang et al., 2020).

Animal studies

In a mice model (*Peromyscus californicus*), exposure to bisphenol A (BPA) (50 mg/kg feed weight) or ethinyl estradiol (0.1 ppb) during periconception and through weaning induced trans-generational and sex-dependent changes on gut microbiota. Proportions of the different genus were affected in fecal samples of parental (P0) and progeny (F1) populations, linked to shifts in metabolic and behavioral traits. Altered bacteria by BPA or EE exposure (e.g., *Bacteroides*, *Mollicutes*, *Prevotellaceae*, *Erysipelotrichaceae*, *Akkermansia*, *Methanobrevibacter*, *Sutterella*) have been previously associated with different disorders, such as inflammatory bowel disease, metabolic disorders, and colorectal cancer (Rosenfeld, 2017). However, the beneficial *Bifidobacterium*, was also higher in fecal samples of BPA- and EE-exposed F1 females (Javurek et al., 2016). Females exposed to ED showed an enriched increased number of molecules involved in carbohydrate metabolism and synthesis, while males had changes in lysine degradation and phenylalanine and tyrosine metabolism

(Kaur et al., 2020). Thus, the consequences of microbiota shift on health should be further evaluated, together with the translational results.

In C3H/HeN mice, Malaisé et al., 2017 reported that perinatal exposure to BPA (50 µg/kg bw) induced a dysbiotic state in gut microbiota, with a significant reduction in relative abundances of reducing *Bifidobacteria*, *Clostridium butyricum*, and *Clostridium Cluster XIVa*. At day 45 and 170 postnatal, BPA exposed mice had an altered glucose sensitivity, a defect of IgA in fecal content, and immune imbalances. In rabbits, BPA (200 50 µg/kg bw) exposure during mid-gestation and through postnatal day seven also affected gut microbiota by reducing *Oscillospira* and *Ruminococcaceae*. Functional shifts were also observed at intestinal and systemic levels, with a significant drop in short-chain fatty acid production, increased gut permeability, and elevated serum lipopolysaccharide (Reddivari et al., 2017).

Although studies using single toxicants are needed, the exposome comprises hundreds of compounds acting simultaneously as a “chemical cocktail” in which toxicants can interact through antagonistic or synergistic mechanisms (García-Barrera et al., 2012; Van Genderen et al., 2015). This simultaneous exposure to a wide array of chemicals may not display immediate effects, but can have summative consequences over time that result in adverse clinical outcomes several years thereafter (Hernandez et al., 2019; Margina et al., 2019). In that sense, the real real-life risk simulation (RLRS) approach offers an advantage compared to the current regulatory risk assessment approach, which is based on studies administering single chemicals at unrealistically high doses (A. Tsatsakis, 2019; Aristidis Tsatsakis et al., 2019). RLRS integrates data from *in vitro*, *in vivo*, and *in silico* studies, including intermittent, chronic, and combined exposures at variable realistic doses of diverse environmental stressors to better understand their real threat to human health (Am Tsatsakis et al., 2017). Thus, multi-exposure, low-dose, and chronic exposure approaches should be encouraged. However, determination of a lifetime internal exposome is technically and

economically complex, and *in silico* tools could be useful to narrow groups of exposure and chemical analysis (Sabbioni et al., 2020)

Hu et al. reported that adult animals exposed at prenatal, lactation, and post-weaning periods to low doses of diethyl phthalate, methylparaben, triclosan, and their combination, had altered microbiota profiles compared to the control group (Hu et al., 2016). Metagenomic profiling of faeces at the adolescent time showed an increased relative abundance of Bacteroidetes and decreased Firmicutes. Bonvallot et al. used metabolomics to demonstrate that exposure of pregnant rats to low-dose of a mixture of eight pesticides during gestation affects lipid, carbohydrate, and amino-acid metabolism, energy production and storage paths, glucose homeostasis, and oxidative stress in both dams and offspring (Bonvallot et al., 2018). Unfortunately, authors did not analyze the effect of pesticides on the microbiota but observed that hippurate and phenylacetylglutamine, both gut microbiota metabolites, were reduced in the urine of dams (Bonvallot et al., 2018). Thus, changes in metabolic parameters after oral exposure to pesticides may be related to alterations in the structure or function of gut microbiota of dams and/or offspring.

Novel materials pose new challenges to toxicology: nanomaterials and microplastics

Organic and inorganic chemicals have received much attention in environmental and human health monitoring during the last decades. Still, toxicology deals with new challenges associated with novel molecules (e.g., engineered nanomaterial, ENMs) or environmental harms (e.g., microplastics). Both nanoparticles and microplastics can reach the human body through intake of contaminated matrices, as are used in food packaging, which can result from routine food processing or are present in foodstuff and water (Naidu, 2020; Rainieri & Barranco, 2019).

For example, small ZnO nanoparticles (NPs) (13 nm) can cross the intestinal and placental barrier in a murine model after oral exposure, causing fetal developmental toxicity, especially during the organogenesis stage (Teng et al., 2019). The same authors reported a synergistic effect of Cd²⁺ and ZnO NPs co-exposure, with an enhanced maternal-fetal transfer of both pollutants and increased embryotoxicity in mice (Teng et al., 2020), however, no information on gut microbiota was reported.

Regular intake of titanium dioxide (TiO₂) nanoparticles can affect the intestinal mucosa, the liver, the brain, and other internal organs, likely increasing the risk of developing many diseases (reviewed in (Baranowska-Wójcik et al., 2020)). It is plausible that the detrimental health effects could be linked to alterations in gut homeostasis, as TiO₂ altered the release of bacterial metabolites in mice model and promoted the *in vitro* biofilm formation of *Escherichia coli* and *Enterococcus faecalis*, with minimal impact on the composition of the microbiota (Pinget et al., 2019). In an adult rat model, oral exposure to TiO₂ NPs (29 ± 9 nm) at different doses caused alterations in gut microbiota, colonic pathological inflammation, and mitochondrial abnormalities. Increased *L. gasseri*, *Turicibacter*, and *L. NK4A136_group* and decreased *Veillonella* were reported, together with metabolic deregulation and intestinal oxidative stress (Chen et al., 2019). Thus, available data suggest microbiota-mediated effects of ENMs on human health, via direct antimicrobial properties towards gut microorganisms or through structural or functional dysbiosis induction, which is subsequently related to intestinal inflammation, metabolic disorders, or immunological changes (reviewed in (Pietroiusti et al., 2016)).

Another contaminant group with increased interest during the last years is microplastics (MPs) (box 1). Intake of MPs has been estimated in the US general population between 39-50 x 10³ microparticles per year, with increased values when considering inhalation sources or intake of bottled water (Cox et al., 2019; Mason et al., 2018). Polypropylene MPs were

consistently detected in human stool samples, although low participant numbers ($n = 8$) can hamper the relevance of this study (Schwabl et al., 2019). In animal models, MPs can interact with the gut microbiota, causing dysbiosis, metabolic disorders, and alterations in the immune system (Lu et al., 2019). MPs induced inflammation of the small intestine, induced IL-1 α in serum, and decreased the Th17 and Treg cells among CD4⁺ cells in mice model (Li et al., 2020). Maternal exposure to MPs also increased the risk of alterations in fatty acid metabolism in offspring, with significant changes in hepatic markers, serum triglycerides, and cholesterol levels in F1 mice progeny (Luo et al., 2019). These effects could be linked to alteration in gut microbiota, but a causal role still needs to be established.

These findings highlight the need to better understand the physiological and developmental effect of engineered nanomaterials and microplastics, especially on the interplay between maternal and neonatal microbiome. Monitoring studies in human populations are encouraged.

Ingredients in food processing: so soft, so sweet...but so healthy?

Emulsifiers

Accepted ingredients in the food industry can also be considered as xenobiotics (box 1). Recently, a randomized controlled-feeding study in healthy adults ($n = 16$) showed alterations in gut microbiota between placebo and carboxymethylcellulose (CMC) groups. Participants in the CMC group also reported increased abdominal pain compared to the non-CMC group. Although these results are novel, the high interindividual variability and a low number of participants could require further confirmation (Chassaing et al., 2020). In animal studies, low doses of emulsifiers (CMC and/or polysorbate-80) induced body weight gain, low-grade inflammation, and metabolic disorders in mice through changes in the colonic and small intestinal microbiota, affecting the intestinal permeability and promoting a proinflammatory environment (Chassaing et al., 2015, 2017; Furuhashi et al., 2020; Viennois et al., 2017). In

mice a model, Nejrup et al., 2017 observed differential effects on the establishment of gut microbiota in mice when different fat types and emulsifiers were administered (Nejrup et al., 2017). Fat and emulsifiers, mainly long-chained fatty acids emulsified with dairy proteins and soy lecithin in infant formula, differ from natural breast-milk lipids (medium-chained fatty acids and triacylglycerols emulsified by a sphingomyelin-rich phospholipid membrane) (Nejrup et al., 2017).

To our knowledge, there is no evidence on the possible impact of maternal intake of emulsifiers on fetal and infant health and vertical microbiota transference in humans. Still, it is plausible that the effects of food emulsifiers on maternal microbiota and chronic low-grade inflammation could transfer specific signatures to newborns (Fink et al., 2019). The role of infant formula emulsifiers on gut microbiota engraftment, intestinal epithelial barrier, and innate immune priming is not yet evaluated.

High- intensity sweeteners

Another group of ingredients with increased consumption during the last years are synthetic non-nutritive sweeteners (NNS). During the last years, increased trends in NNS consumption, including child consumption, has been described, likely due to marketing of NNS as healthy ingredients to substitute sugar consumption and prevent obesity pandemic (Archibald et al., 2018). In humans, maternal intake of NNS sucralose and acesulfame-K during pregnancy was associated with higher infant BMI (reviewed in (Archibald et al., 2018), and it has been described that both compounds transfer into breast milk following ingestion (Rother et al., 2018). Sylvestsky et al. detected NNS (sucralose, acesulfame-K, and saccharin) in breastmilk of 65% of lactating women (n = 20) (Sylvestsky et al., 2015). In adult human populations and animal models, the effect of sweeteners on the gut microbiota has been extensively demonstrated (reviewed or commented in (Y. Cao et al., 2020; Ruiz-Ojeda et al., 2019;

Schiffman & Nagle, 2019)), but human data of the effect of NNS on maternal-infant microbiota are still scarce. Recently, a human study, including 100 infants from the prospective Canadian CHILD Cohort Study showed an impact of maternal consumption of artificially sweetened beverages during pregnancy and infant gut microbiota and BMI at 12 months. Remarkably, shifts in microbial communities included depletion of *Bacteroides* spp., and specifically in a subgroup of infants, an enrichment in *Akkermansia muciniphila* was observed. These microbiota shifts were associated with higher BMI at one year (Laforest-Lapointe et al., 2020). Without considering the microbiota, NNS exposure during pregnancy has been associated with the increased risk of developing overweight/obesity at 7 years (Yeyi Zhu et al., 2017), child asthma, and allergic rhinitis (Maslova et al., 2013), while long-term consumption of sugar-sweetened and artificially sweetened beverages intake was positively associated with total and cardiovascular disease mortality in a study including 37 716 men (1986-2014) and 80 647 women (1980-2014) in the US population (Malik et al., 2019). Likely, the microbiota can play a role in these observations and precautionary measures to reduce fetal and infant exposure to NNS should be urgently implemented. Mice studies showed that acesulfame-K could cross the placenta resulting in fetal programming of adulthood taste preferences (G.-H. Zhang et al., 2011), the microbiota of the progeny, and metabolic programming (Stichelen et al., 2019). Administration of NNS at representative doses for human consumption to pregnant and lactating mice was associated with drastic metabolic changes in the pups, including alterations of amino acid metabolism, downregulation of hepatic detoxification paths and shifts in bacterial metabolites (Stichelen et al., 2019). These metabolic changes were accompanied by an increased abundance of Firmicutes and a decrease of *Akkermansia muciniphila*. Remarkably, NNS exposed mothers and controls did not show differences in gut microbiota, and *A.muciniphila* was efficiently transferred in both groups, however only engrafting in control pups and not in NNS-exposed

animals (Stichelen et al., 2019). Previous studies in adult mice also showed reductions in *A. muciniphila* after sucralose intake (Suez et al., 2014). The same authors linked glucose intolerance with gut microbiota dysbiosis after artificial sweeteners in adult humans (Suez et al., 2014). Microbial signatures described in NNS-exposed mice have been previously linked to metabolic disease and obesity in humans (Castaner et al., 2018). Besides, *A. muciniphila*, is a degrader mucin microorganism influencing host immune regulation, enhancing the integrity of the intestinal epithelial cells, and the thickness of the mucus layer and having protective effects in diet-induced obesity (Ansaldi et al., 2019; T. Zhang et al., 2019).

Especially during early life, the gut microbiota has strong plasticity and is more sensitive to disruptions on homeostasis. The period comprising from conception to 2 years of age is critical for developmental programming and immune priming. The possible effects on health configuration due to changes occurring in infant microbiota by environmental toxicants and wide-spread food additives are unknown. The time-scale at which environmental pollutants can affect fetal, infant and subsequent development are extended and variable. When exposure to toxicants starts early in life (Figure 1), the cumulative risk (box 1) also needs to be considered, especially for bioaccumulative substances and complex mixtures of toxicants from different nature.

Mother-infant transfer: breastmilk, microbiota and chemicals

Breastfeeding is the gold standard of neonate nutrition, impacting a wide array of health outcomes in both mother and infant (Victora et al., 2016). Human milk composition is dynamic and adaptable to the different stages of development. It contains lipids, peptides, immunoglobulins, human milk oligosaccharides, and growth factors, among other components, which ensure the optimal development of the neonate (Demmelmair et al.,

2020). In addition, microorganisms are also biologically-active elements of the human milk, influencing infant microbial community configurations (Pannaraj et al., 2017).

Several studies have reported residues of organic and inorganic chemicals in breastmilk (Cano-Sancho et al., 2020; Gautam et al., 2020; Pajewska-Szmyt et al., 2019; Park et al., 2018), but less is known about the effect of environmental pollutants on human milk or infant microbiota. In Chinese mothers, increased hexachlorocyclohexane (HCH) levels in colostrum tend to decrease microbial diversity, with the positive association of *Pseudomonas* spp. and HCHs levels (Tang et al., 2019). Iszatt et al., 2019 showed that organic breast milk toxicants affected the gut bacteria composition and short-chain fatty acid profiles in 1 month-infants (Iszatt et al., 2019), however, breastmilk microbiota was not assessed. Linking both studies, infants' exposure to xenobiotics through colostrum and breastmilk can significantly affect the establishment of early-life microbiota and immune priming. *Pseudomonas* spp. has been described as a major component of the human milk microbiota, together with *Staphylococcus*, *Streptococcus*, and *Acinetobacter* (Boix-Amorós et al., 2016). The link between HCH residues in milk and *Pseudomonas*' increase could be associated to the degrading-capacity of HCH (Tang et al., 2019). *Pseudomonas aeruginosa* elastase can cleave human colostral IgA (Heck et al., s. f.); thus, colostrum or milk microbiota alterations can have unknown consequences on infants' health programming.

Future perspectives

Microbiota-host interplay during early life is a critical factor in health programming, but little is known on the interaction between diet-xenobiotics-microbiota during pregnancy and perinatal period, and the consequences for infant and adult health. However, the current evidence shows that maternal-infant dyad microbiota can be a sensitive target of xenobiotics exposure. During critical periods of development, exposure to contaminants from food may

have dysbiotic effects on the establishment and maturation of the human microbiota, having long-lasting consequences on metabolic and immune homeostasis.

Standardized *in vitro* and *in vivo* translational models, including microbiota, should be established in parallel with observational studies to obtain simultaneously human-based evidence data and mechanistic understanding of xenobiotics' mode of action. This approach may speed up our knowledge of the long-term and transgenerational effects of chemical exposures.

The presence of new environmental contaminants such as microplastics may interfere with certain pollutants' distribution and fate; therefore, a better characterization of toxic effects of prenatal and early postnatal exposure to these compounds should be addressed, mainly focusing on gut microbiota acquisition and immune priming.

New challenges on toxicant's exposure assessment include the development of economically affordable and sensitive analytical techniques for multi-elemental quantification, as well as accurate detection of novel xenobiotics or metabolites. The multi-factorial origin of diseases should account for a comprehensive view of environmental, genetic, and psychological factors affecting infant health, including mixed toxicants exposures. Incorporating omic and bioinformatics tools to decipher the interplay between toxicants, infant microbiota, and health outcomes will give scientific support to educational, dietary, or other intervention schemes to reduce disease burden the future generations and safeguard consumer's health. Translation of science-based results to stakeholders may require careful examination, looking for faster paths which ensure preventive strategies are implemented on time (Grandjean et al., 2019).

Conclusions

583 *In utero* and early-life are critical periods for human development and health programming. A
584 proper establishment and development of the human microbiota are closely related to
585 immune and metabolic health, and microbiota acquisition or development disruptions have
586 been linked to several pathological conditions. A growing body of evidence shows that
587 multiple xenobiotics from different groups (organic, inorganics), including specific food
588 additives (e.g., artificial sweeteners and emulsifiers), are in contact with pregnant women or
589 mothers and are consequently transferred to the fetus, neonates, or infants, having
590 consequences on gut microbiota structure and function, and hence impairing immune
591 signalling and metabolic control. Abundances of health-associated bacteria belonging to
592 *Bifidobacterium*, *Lactobacillus*, *Ruminococcus* or *Akkermansia* genus seem to be sensitive to
593 xenobiotic exposure. Xenobiotic-induced dysbiosis in breastmilk and maternal-infant gut
594 microbiota can play a role in the increased prevalence of specific pathologies as obesity,
595 diabetes, or asthma. Still, currently, most of our current knowledge is based on a limited
596 number of human and animal studies. Thus, infants are particularly vulnerable to
597 environmental exposures putting them at a high level of ambient risk. Including microbiota as
598 a player in exposure and risk assessment is urgently required, especially during critical
599 windows of susceptibility as pre-, peri- and neonatal periods and infancy. The interplay
600 between dietary components, nutritional deficits, or other environmental or psychosocial
601 factors affecting the microbiota will require validated protocols, mechanistic approaches, and
602 observational studies running in parallel to ensure the relevance of the outcomes. More
603 research is required, including multi and interdisciplinary approaches considering omic
604 technologies and efficient big data processing. The challenge of assessing microbiota effects
605 on life span, multi-xenobiotic exposure will require the scientific community's effort and
606 conscious involvement of stakeholders. However, in the meantime, it is recommended to

607 apply the principle of precaution, especially in pregnant women and infant populations,
608 priming consumer's health versus any other socioeconomic factor.

609
610
611 **BOX 1. Terms and definitions**

612 Xenobiotic: chemicals to which an organism is exposed that are extrinsic to the normal
613 metabolism of that organism (Croom, 2012)

614 Exposome: concept describing the totality of exposure experienced by an individual during
615 their life and the health impact of those exposures (DeBord et al., 2016)

616 Microbiota: the assemblage of microorganisms present in a defined environment (Marchesi &
617 Ravel, 2015)

618 Epigenetic: influence of the developmental environment on the mature phenotype. Structural
619 changes to genes without altering the nucleotide sequence (Godfrey et al., 2007)

620 Cumulative risk: combined hazard resulting from exposures that accumulate over time,
621 pathways, sources, or routes for multiple agents or stressors (WHO, 2006)

622 Microplastics: any synthetic solid particle or polymeric matrix, with regular or irregular shape
623 and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing
624 origin, which are insoluble in water (Frias & Nash, 2019)

626

627 Figure 1. Schematic representation of factors affecting the establishment of the human microbiota and the interplay with xenobiotics. The maternal role in
 628 modifying primary xenobiotics by microbial or hepatic metabolism can alter the exposure levels and chemical species on fetus or neonate (maternal "filter").
 629 The lifetime exposure to contaminants influences epigenetic traits, and microbiota signatures could have a transgenerational effect.

630 **Table 1.** Exposure to environmental toxicants during pre- and early postnatal periods and the effects on gut microbiota. Selected references from
631 2017-2020, including microbiota and toxicant (metals, metalloids, persistent organic pollutants) analysis in human epidemiological studies, were
632 included.

Population	Sample analyzed	Analysis	Effects	Ref.
Inorganic compounds				
Wayne County Health, Environment, Allergy and Asthma Longitudinal Study (WHEALS) (2003-2007) and WHEALS Tooth Fairy Study (2011-2019)	N=146 Pb in tooth Stool samples (1 and 6 months of age)	Pb levels in tooth 16S rRNA ITS2 sequencing	↑Pb (2nd and 3 rd trimester) ↑ <i>Collinsella</i> abundance at 1 month and ↑ <i>Biophila</i> at 6 months ↑Pb in utero and postnatal ↓ <i>Bacteroides</i> OTUs at 1 and 6 months ↑Pb (2nd and 3 rd trimester) ↓ <i>Candida</i> and <i>Aspergillus</i> and ↑ <i>Malassezia</i> and <i>Saccharomyces</i>	(Sitarik et al., 2020)
Prospective birth cohort Bangladeshi (2007–2011)	N= 50 children, 25 with fetal and early-life exposure to high levels of As (> 50 µg/L As in water) 4-6 years' old	As in drinking water 16S rRNA whole metagenome shotgun sequencing	↑As ↑abundance of Proteobacteria phylum, class Gammaproteobacteria, order Enterobacteriales, family Enterobacteriaceae ↑As ↑ <i>E.coli</i> ↑As ↑ antibiotic-resistance genes (soxS, marA, cpxR)	(Dong et al., 2017)
New Hampshire Birth Cohort Study (2009-actual).	N=204 infants Infant urine and fecal sample 6-week old	Total As in urine 16S rRNA	Eight genera, six within the phylum Firmicutes, were enriched with higher As exposure. Fifteen genera were negatively associated with urinary As concentration, including <i>Bacteroides</i> and <i>Bifidobacterium</i> . Upon stratification by both sex and feeding method, detectable associations among formula-fed males (p = 0.008), but not other groups (p > 0.05) for formula-fed females and for breastfed males and females)	(Hoen et al., 2018)
Mothers recruited for the study in Charleston, South Carolina, USA	Maternal blood and stool samples Hair THg Meconium Early gestation (8.3-17 wk) n=28	Total mercury and MeHg concentrations in early (8.3–17 weeks,) and late (27–36 weeks, n=24) gestation	54% of the correlations between phyla and Hg parameters changed direction of association between early and late gestation. Early gestation: Associations	(Rothenberg et al., 2016)

(2014-2016)	Late gestation (2736 wk) n=24	16S rRNA (n = 52)	(positive/negative) between Hg parameters and <i>Lachnospiraceae</i> , <i>Collinsella</i> , <i>Ruminococcaceae_ucg002</i> , <i>Ruminococcaceae_ucg013</i> , <i>Prevotella_9</i> ↑ Hg parameters ↑ <i>Faecalibacterium</i> , <i>Lachnospiraceae_nk4a136</i> , <i>Megasphaera</i> , <i>Ruminococcaceae_ucg013</i> , and <i>Parabacteroides</i>	
Fetal and infant exposure New Hampshire Birth Cohort Study (2009-actual)	179 infants, 6-week old Infant toenail clippings and fecal sample	Metal(oid)s (Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, Sb, Hg, Pb) 16S rRNA	↑ arsenic+antibiotics) ↓ microbial diversity ↑ Zinc ↓ <i>Bifidobacterium</i> Quadratic relationship between zinc and arsenic	(Laue et al., 2020)
Organic compounds				
GESTation and Environment (GESTE) longitudinal birth cohort (2007-2009)	Maternal plasma during pregnancy Pregnancy, n = 9; delivery n = 9 Infant (6-8 years) fecal sample	PBDEs and PCBs PBDE-47, -99, -100, and -153 and PCB-138, -153, and -180 in maternal plasma 16S rRNA	Changes in infant gut microbiota ↑ PCB-153, PCB-180, Σ3PCB ↑ Propionibacteriales and Propionibacteriaceae ↑ PCB-180 and Σ3PCB ↑ Bacillales Family XI. ↑ PBDE-99 ↓ Ruminococcaceae ↑ PBDE-47 differences in <i>Ruminococcus 2</i> .	(Laue et al., 2019)
Norwegian Micorbiota Cohort (NoMIC) (2002-2005)	267 mother-child pairs 1 month Breastmilk and infant fecal sample	28 chemical exposures: PCBs, PBDEs, PFASs, and organochlorine pesticides 16S rRNA	↑ PBDE-28 and PFOS ↓ neonatal microbiome diversity ↑ toxicant exposure ↓ <i>Lactobacillus</i> genus ↑ PBDE-28 in breast milk ↓ propionic acid in infant fecal sample ↑ PCB-209 in breast milk ↓ acetic acid in infant fecal sample ↑ PFOA in breast milk ↑ propionic acid ↑ PCB-167 in breast milk ↑ acetic acid	(Iszatt et al., 2019)

633 Pb: Lead; As: Arsenic; PBDEs: Polybrominated Diphenyl Ethers (PBDEs); PCBs: polychlorinated biphenyls (PCBs); PFOS:perfluorooctanesulfonic acid;
634 total mercury: THg. 16S rRNA gene profiling was performed in fecal samples, otherwise stated.

**BOX 2. Critical questions and gaps in the assessment of the interplay between
contaminants and maternal-infant microbiota**

- Are we using the appropriate biomarkers of exposure and toxicity? Is it the stability of the parental compounds and metabolites enough for assessing the real exposures? Where are we evaluating the exposure: serum, urine, blood, hair, nails, feces? How can long-term fetal exposure be quantitatively assessed?
- Are the toxicity endpoints directly related to the gut microbiota? Are other factors affecting the gut microbiota interfering with the findings? (e.g., food interactions)
- How xenobiotic's exposure affects other microbial niches (e.g., lung, skin)?
- Is it the susceptibility window well-chosen? Are we taking the snapshot at the proper time?
- Are we evaluating healthy or diseased populations? Do we know what healthy or diseased gut microbiota is?
- What is the effect of maternal exposure to environmental pollutants on the placenta, amniotic fluid, and blood cord microbiota, if existing, and how this could affect immune priming, fetal development, and health of future generations?
- Is it the co-occurrence of several environmental pollutants and nutritional deficits or other factors affecting metabolism, gut microbiota functionality, or host well-being (e.g., psychological stress) considered in current studies?
- Are the animal models good enough to achieve translational results?
- How can we improve *in vivo/in vitro* models to accurately assess the mode of action and endpoints for long-term risk assessment from early-life exposures?

Funding sources

Collado M. and Calatayud Arroyo M acknowledge the support from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (ERC starting grant, n° 639226) and the Project from The Ramón Areces Foundation (ref. CIVP19A5918). García Barrera T., Callejón Leblic B. and Arias Borrego A. have been supported by the projects UHU-1256905 from the FEDER Andalusian operative program (Regional Ministry of Economy, Knowledge, Business and University, Andalusia, Spain) and PGC2018-096608-B-C21 from the Spanish Ministry of Economy and Competitiveness (MINECO).

Disclosure of potential conflicts of interest

The authors report no potential conflict of interest.

REFERENCES

1. Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science translational medicine*, 6(237), 237ra65-237ra65. DOI: 10.1126/scitranslmed.3008599
2. Abdelsalam, N. A., Ramadan, A. T., ElRakaiby, M. T., & Aziz, R. K. (2020). Toxicomicrobiomics: The Human Microbiome vs. Pharmaceutical, Dietary, and Environmental Xenobiotics. *Frontiers in Pharmacology*, 11, 390. DOI: 10.3389/fphar.2020.00390
3. Adams, R. I., Bateman, A. C., Bik, H. M., & Meadow, J. F. (2015). Microbiota of the indoor environment: A meta-analysis. *Microbiome*, 3(1), 49. <https://doi.org/10.1186/s40168-015-0108-3>
4. Ansaldo, E., Slayden, L. C., Ching, K. L., Koch, M. A., Wolf, N. K., Plichta, D. R., Brown, E. M., Graham, D. B., Xavier, R. J., & Moon, J. J. (2019). *Akkermansia muciniphila* induces intestinal adaptive immune responses during homeostasis. *Science*, 364(6446), 1179-1184. DOI: 10.1126/science.aaw7479
5. Archibald, A. J., Dolinsky, V. W., & Azad, M. B. (2018). Early-life exposure to non-nutritive sweeteners and the developmental origins of childhood obesity: Global evidence from human and rodent studies. *Nutrients*, 10(2), 194. DOI: 10.3390/nu10020194
6. Askarova, S., Umbayev, B., Masoud, A.-R., Kaiyrlykzy, A., Safarova, Y., Tsoy, A., Olzhayev, F., & Kushugulova, A. (2020). The Links Between the Gut Microbiome, Aging, Modern Lifestyle and Alzheimer's Disease. *Frontiers in Cellular and Infection Microbiology*, 10, 104. DOI: 10.3389/fcimb.2020.00104
7. Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., & Zhong, H. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell host & microbe*, 17(5), 690-703. DOI: 10.1016/j.chom.2015.04.004
8. Bailey, J. L., Dalvai, M., Lessard, M., Herst, P. M., Charest, P. L., & Navarro, P. (2020). Beyond fertilisation: How the paternal environment influences future generations. *Animal Reproduction Science*, 106503. DOI: 10.1016/j.anireprosci.2020.106503
9. Baillie-Hamilton, P. F. (2002). Chemical toxins: A hypothesis to explain the global obesity epidemic. *The journal of alternative & complementary medicine*, 8(2), 185-192. DOI: 10.1089/107555302317371479
10. Baranowska-Wójcik, E., Sz wajgier, D., Oleszczuk, P., & Winiarska-Mieczan, A. (2020). Effects of titanium dioxide nanoparticles exposure on human health—A review. *Biological Trace Element Research*, 193(1), 118-129. DOI: 10.1007/s12011-019-01706-6
11. Barker, D. J. (2007). The origins of the developmental origins theory. *Journal of internal medicine*, 261(5), 412-417. DOI: 10.1111/j.1365-2796.2007.01809.x
12. Boix-Amorós, A., Collado, M. C., & Mira, A. (2016). Relationship between Milk Microbiota, Bacterial Load, Macronutrients, and Human Cells during Lactation. *Frontiers in Microbiology*, 7. DOI: 10.3389/fmicb.2016.00492
13. Bonvallot, N., Canlet, C., Blas-Y-Estrada, F., Gautier, R., Tremblay-Franco, M., Chevolleau, S., Cordier, S., & Cravedi, J.-P. (2018). Metabolome disruption of pregnant rats and their offspring resulting from repeated exposure to a pesticide mixture representative of environmental contamination in Brittany. *PloS one*, 13(6), e0198448. DOI: 10.1371/journal.pone.0198448
14. Cano-Sancho, G., Alexandre-Gouabau, M.-C., Moyon, T., Royer, A.-L., Guitton, Y., Billard, H., Darmaun, D., Rozé, J.-C., Boquien, C.-Y., & Le Bizec, B. (2020). Simultaneous exploration of nutrients and pollutants in human milk and their impact on preterm infant growth: An integrative cross-platform approach. *Environmental Research*, 182, 109018. DOI: 10.1016/j.envres.2019.109018
15. Cao, B., Stout, M. J., Lee, I., & Mysorekar, I. U. (2014). Placental microbiome and its role in preterm birth. *Neoreviews*, 15(12), e537-e545. DOI: doi.org/10.1542/neo.15-12-e537

16. Cao, Y., Liu, H., Qin, N., Ren, X., Zhu, B., & Xia, X. (2020). Impact of food additives on the composition and function of gut microbiota: A review. *Trends in Food Science & Technology*. DOI: 10.1016/j.tifs.2020.03.006
17. Castaner, O., Goday, A., Park, Y.-M., Lee, S.-H., Magkos, F., Shioh, S.-A. T. E., & Schröder, H. (2018). The gut microbiome profile in obesity: A systematic review. *International journal of endocrinology*, 2018. DOI: 10.1155/2018/4095789
18. CEFIC. (2020). Facts and Figures of the European Chemical Industry. <https://cefic.org/app/uploads/2019/01/The-European-Chemical-Industry-Facts-And-Figures-2020.pdf>
19. Chassaing, B., Gershuni, V., McKeever, L., Compher, C., Bonhomme, B., Harb, A. H., Nessel, L., Wu, G. D., Gewirtz, A. T., & Lewis, J. D. (2020). 1008 A RANDOMIZED CONTROLLED-FEEDING STUDY OF THE IMPACT OF THE SYNTHETIC DIETARY EMULSIFIER CARBOXYMETHYLCELLULOSE ON HUMAN HEALTH AND INTESTINAL MICROBIOTA. *Gastroenterology*, 158(6), S-200. DOI: 10.1016/S0016-5085(20)31184-7
20. Chassaing, B., Koren, O., Goodrich, J. K., Poole, A. C., Srinivasan, S., Ley, R. E., & Gewirtz, A. T. (2015). Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature*, 519(7541), 92-96. DOI: 10.1038/nature14232
21. Chassaing, B., Van de Wiele, T., De Bodt, J., Marzorati, M., & Gewirtz, A. T. (2017). Dietary emulsifiers directly alter human microbiota composition and gene expression ex vivo potentiating intestinal inflammation. *Gut*, 66(8), 1414-1427. DOI: 10.1136/gutjnl-2016-313099
22. Chen, Z., Han, S., Zhou, D., Zhou, S., & Jia, G. (2019). Effects of oral exposure to titanium dioxide nanoparticles on gut microbiota and gut-associated metabolism in vivo. *Nanoscale*, 11(46), 22398-22412. DOI: 10.1039/C9NR07580A
23. Christoff, A. P., Sereia, A. F., Hernandez, C., & de Oliveira, L. F. (2019). Uncovering the hidden microbiota in hospital and built environments: New approaches and solutions. *Experimental Biology and Medicine*, 244(6), 534-542. DOI: 10.1177/1535370218821857
24. Chu, D., Stewart, C., Seferovic, M., Suter, M., Cox, J., Vidaeff, A., & Aagaard, K. (2017). 26: Profiling of microbiota in second trimester amniotic fluid reveals a distinctive community present in the mid trimester and predictive of the placental microbiome at parturition. *American Journal of Obstetrics & Gynecology*, 216(1), S18-S19. DOI: 10.1016/j.ajog.2016.11.917
25. Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Scientific reports*, 6, 23129. DOI: 10.1038/srep23129
26. Collins, J. K., Thornton, G., & Sullivan, G. O. (1998). Selection of Probiotic Strains for Human Applications. *International Dairy Journal*, 8(5-6), 487-490. DOI: 10.1016/S0958-6946(98)00073-9
27. Collins, S. L., & Patterson, A. D. (2020). The gut microbiome: An orchestrator of xenobiotic metabolism. *Acta Pharmaceutica Sinica B*, 10(1), 19-32. DOI: 10.1016/j.apsb.2019.12.001
28. Colliou, N., Ge, Y., Sahay, B., Gong, M., Zadeh, M., Owen, J. L., Neu, J., Farmerie, W. G., Alonzo, F., & Liu, K. (2017). Commensal *Propionibacterium* strain UF1 mitigates intestinal inflammation via Th17 cell regulation. *The Journal of clinical investigation*, 127(11), 3970-3986. DOI: 10.1172/JCI95376
29. Cox, K. D., Covernton, G. A., Davies, H. L., Dower, J. F., Juanes, F., & Dudas, S. E. (2019). Human consumption of microplastics. *Environmental science & technology*, 53(12), 7068-7074. DOI: 10.1021/acs.est.9b01517
30. Croom, E. (2012). Metabolism of xenobiotics of human environments. *En Progress in molecular biology and translational science* (Vol. 112, pp. 31-88). Elsevier. DOI: 10.1016/B978-0-12-415813-9.00003-9

31. Dai, J., Yang, X., Yuan, Y., Jia, Y., Liu, G., Lin, N., Xiao, H., Zhang, L., & Chen, J. (2020). Toxicity, gut microbiota and metabolome effects after copper exposure during early life in SD rats. *Toxicology*, 433, 152395. DOI: 10.1016/j.tox.2020.152395
32. de Goffau, M. C., Lager, S., Sovio, U., Gaccioli, F., Cook, E., Peacock, S. J., Parkhill, J., Charnock-Jones, D. S., & Smith, G. C. (2019). Human placenta has no microbiome but can contain potential pathogens. *Nature*, 572(7769), 329-334. DOI: 10.1038/s41586-019-1451-5
33. DeBord, D. G., Carreón, T., Lentz, T. J., Middendorf, P. J., Hoover, M. D., & Schulte, P. A. (2016). Use of the “exposome” in the practice of epidemiology: A primer on-omic technologies. *American journal of epidemiology*, 184(4), 302-314. DOI: 10.1093/aje/kwv325
34. Demmelmair, H., Jiménez, E., Collado, M. C., Salminen, S., & McGuire, M. K. (2020). Maternal and perinatal factors associated with the human milk microbiome. *Current Developments in Nutrition*, 4(4). DOI: 10.1093/cdn/nzaa027
35. Dietert, R. R., & Silbergeld, E. K. (2015). Biomarkers for the 21st century: Listening to the microbiome. *Toxicological Sciences*, 144(2), 208-216. DOI: 10.1093/toxsci/kfv013
36. Dominguez-Bello, M. G., Godoy-Vitorino, F., Knight, R., & Blaser, M. J. (2019). Role of the microbiome in human development. *Gut*, 68(6), 1108-1114. DOI: 10.1136/gutjnl-2018-317503
37. Dong, X., Shulzhenko, N., Lemaitre, J., Greer, R. L., Peremyslova, K., Quamruzzaman, Q., Rahman, M., Hasan, O. S. I., Joya, S. A., & Golam, M. (2017). Arsenic exposure and intestinal microbiota in children from Sirajdikhan, Bangladesh. *PLoS One*, 12(12), e0188487.
38. EPA. (2020). TSCA Chemical Substance Inventory. <https://www.epa.gov/tsca-inventory/how-access-tsca-inventory>
39. Ferretti, P., Pasoli, E., Tett, A., Asnicar, F., Gorfer, V., Fedi, S., Armanini, F., Truong, D. T., Manara, S., Zolfo, M., Beghini, F., Bertorelli, R., De Sanctis, V., Bariletti, I., Canto, R., Clementi, R., Cologna, M., Crifò, T., Cusumano, G., ... Segata, N. (2018). Mother-to-Infant Microbial Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. *Cell Host & Microbe*, 24(1), 133-145.e5. DOI: 10.1016/j.chom.2018.06.005
40. Fink, N. R., Chawes, B., Bønnelykke, K., Thorsen, J., Stokholm, J., Rasmussen, M. A., Brix, S., & Bisgaard, H. (2019). Levels of systemic low-grade inflammation in pregnant mothers and their offspring are correlated. *Scientific reports*, 9(1), 1-9. DOI: 10.1038/s41598-019-39620-5
41. Frias, J. P. G. L., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. *Marine Pollution Bulletin*, 138, 145-147. DOI: 10.1016/j.marpolbul.2018.11.022
42. Furuhashi, H., Higashiyama, M., Okada, Y., Kurihara, C., Wada, A., Horiuchi, K., Hanawa, Y., Mizoguchi, A., Nishii, S., Inaba, K., Sugihara, N., Watanabe, C., Komoto, S., Tomita, K., Miura, S., & Hokari, R. (2020). Dietary emulsifier polysorbate-80-induced small-intestinal vulnerability to indomethacin-induced lesions via dysbiosis. *Journal of Gastroenterology and Hepatology*, 35(1), 110-117. DOI: 10.1111/jgh.14808
43. Gao, J., Li, Y., Wan, Y., Hu, T., Liu, L., Yang, S., Gong, Z., Zeng, Q., Wei, Y., & Yang, W. (2019). A novel postbiotic from *Lactobacillus rhamnosus* GG with a beneficial effect on intestinal barrier function. *Frontiers in microbiology*, 10, 477. DOI: 10.3389/fmicb.2019.00477
44. García-Barrera, T., Gómez-Ariza, J. L., González-Fernández, M., Moreno, F., García-Sevillano, M. A., & Gómez-Jacinto, V. (2012). Biological responses related to agonistic, antagonistic and synergistic interactions of chemical species. *Analytical and bioanalytical chemistry*, 403(8), 2237-2253. DOI: 10.1007/s00216-012-5776-2
45. Gaucher, F., Bonnassie, S., Rabah, H., Marchand, P., Blanc, P., Jeantet, R., & Jan, G. (2019). Adaptation of beneficial *Propionibacteria*, *Lactobacilli*, and *Bifidobacteria* improves tolerance toward technological and digestive stresses. *Frontiers in microbiology*, 10, 841. DOI: 10.3389/fmicb.2019.00841

46. Gaulke, C. A., Rolshoven, J., Wong, C. P., Hudson, L. G., Ho, E., & Sharpton, T. J. (2018). Marginal zinc deficiency and environmentally relevant concentrations of arsenic elicit combined effects on the gut microbiome. *mSphere*, 3(6). DOI: 10.1128/mSphere.00521-18
47. Gautam, B., Rogge, M. M., Acharya, N., Keesari, R., & Almekdash, M. H. (2020). Obesogenic Toxicants in Breast Milk of Lactating Women: Investigation of a Risk Factor for Childhood Obesity. *Biological Research For Nursing*, 22(2), 295-301. DOI: 10.1177/1099800420909151
48. Godfrey, K. M., Lillycrop, K. A., Burdge, G. C., Gluckman, P. D., & Hanson, M. A. (2007). Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. *Pediatric research*, 61(7), 5-10. DOI: 10.1203/pdr.0b013e318045bedb
49. González, N., Marquès, M., Nadal, M., & Domingo, J. L. (2019). Occurrence of environmental pollutants in foodstuffs: A review of organic vs. conventional food. *Food and Chemical Toxicology*, 125, 370-375. DOI: 10.1016/j.fct.2019.01.021
50. Goodrich, J. M., Ingle, M. E., Domino, S. E., Treadwell, M. C., Dolinoy, D. C., Burant, C., Meeker, J. D., & Padmanabhan, V. (2019). First trimester maternal exposures to endocrine disrupting chemicals and metals and fetal size in the Michigan Mother Infant Pairs Study. *Journal of developmental origins of health and disease*, 10(4), 447. DOI: 10.1017/S204017441800106X
51. Grandjean, P., Abdennebi-Najar, L., Barouki, R., Cranor, C. F., Etzel, R. A., Gee, D., Heindel, J. J., Hougaard, K. S., Hunt, P., & Nawrot, T. S. (2019). Timescales of developmental toxicity impacting on research and needs for intervention. *Basic & clinical pharmacology & toxicology*, 125, 70-80. DOI: 10.1111/bcpt.13162
52. Heck, L. W., Alarcon, P. G., Kulhavy, R. M., Morihara, K., & Mestecky, J. F. (s. f.). Degradation of IgA proteins by *Pseudomonas aeruginosa* elastase. 6.
53. Heindel, J. J. (2020). Long-Term Implications of Developmental Programming and Obesity. In *Health Impacts of Developmental Exposure to Environmental Chemicals* (pp. 191-220). Springer. Online ISBN 978-981-15-0520-1
54. Heindel, J. J., Balbus, J., Birnbaum, L., Brune-Drisse, M. N., Grandjean, P., Gray, K., Landrigan, P. J., Sly, P. D., Suk, W., & Slechta, D. C. (2015). Developmental origins of health and disease: Integrating environmental influences. *Endocrinology*, 156(10), 3416-3421. DOI: 10.1210/EN.2015-1394
55. Hernandez, A. F., Buha, A., Constantin, C., Wallace, D. R., Sarigiannis, D., Neagu, M., Antonijevic, B., Hayes, A. W., Wilks, M. F., & Tsatsakis, A. (2019). Critical assessment and integration of separate lines of evidence for risk assessment of chemical mixtures. *Archives of toxicology*, 93(10), 2741-2757. DOI: 10.1007/s00204-019-02547-x
56. Hoen, A. G., Madan, J. C., Li, Z., Coker, M., Lundgren, S. N., Morrison, H. G., Palys, T., Jackson, B. P., Sogin, M. L., & Cottingham, K. L. (2018). Sex-specific associations of infants' gut microbiome with arsenic exposure in a US population. *Scientific reports*, 8(1), 1-10. DOI: 10.1038/s41598-018-30581-9
57. Hu, J., Raikhel, V., Gopalakrishnan, K., Fernandez-Hernandez, H., Lambertini, L., Manservigi, F., Falcioni, L., Bua, L., Belpoggi, F., & Teitelbaum, S. L. (2016). Effect of postnatal low-dose exposure to environmental chemicals on the gut microbiome in a rodent model. *Microbiome*, 4(1), 1-11. DOI: 10.1186/s40168-016-0173-2
58. Indiani, C. M. dos S. P., Rizzardi, K. F., Castelo, P. M., Ferraz, L. F. C., Darrieux, M., & Parisotto, T. M. (2018). Childhood Obesity and Firmicutes/Bacteroidetes Ratio in the Gut Microbiota: A Systematic Review. *Childhood Obesity*, 14(8), 501-509. DOI: 10.1089/chi.2018.0040
59. Iszatt, N., Janssen, S., Lenters, V., Dahl, C., Stigum, H., Knight, R., Mandal, S., Peddada, S., González, A., & Midtvedt, T. (2019). Environmental toxicants in breast milk of Norwegian mothers and gut bacteria composition and metabolites in their infants at 1 month. *Microbiome*, 7(1), 34. DOI: 10.1186/s40168-019-0645-2

60. Javurek, A. B., Spollen, W. G., Johnson, S. A., Bivens, N. J., Bromert, K. H., Givan, S. A., & Rosenfeld, C. S. (2016). Effects of exposure to bisphenol A and ethinyl estradiol on the gut microbiota of parents and their offspring in a rodent model. *Gut microbes*, 7(6), 471-485. DOI: 10.1080/19490976.2016.1234657
61. Jin, Y., Wu, S., Zeng, Z., & Fu, Z. (2017). Effects of environmental pollutants on gut microbiota. *Environmental Pollution*, 222, 1-9. DOI: 10.1016/j.envpol.2016.11.045
62. Kaur, S., Sarma, S. J., Marshall, B. L., Liu, Y., Kinkade, J. A., Bellamy, M. M., Mao, J., Helferich, W. G., Schenk, A. K., Bivens, N. J., Lei, Z., Sumner, L. W., Bowden, J. A., Koelmel, J. P., Joshi, T., & Rosenfeld, C. S. (2020). Developmental exposure of California mice to endocrine disrupting chemicals and potential effects on the microbiome-gut-brain axis at adulthood. *Scientific Reports*, 10(1), 10902. DOI: 10.1038/s41598-020-67709-9
63. Kerr, C. A., Grice, D. M., Tran, C. D., Bauer, D. C., Li, D., Hendry, P., & Hannan, G. N. (2015). Early life events influence whole-of-life metabolic health via gut microflora and gut permeability. *Critical reviews in microbiology*, 41(3), 326-340. DOI: 10.3109/1040841X.2013.837863
64. Koppel, N., Rekdal, V. M., & Balskus, E. P. (2017). Chemical transformation of xenobiotics by the human gut microbiota. *Science*, 356(6344). DOI: 10.1126/science.aag2770
65. Kostoff, R. N., Goumenou, M., & Tsatsakis, A. (2018). The role of toxic stimuli combinations in determining safe exposure limits. Elsevier. DOI: 10.1016/j.toxrep.2018.10.010
66. Laforest-Lapointe, I., Becker, A. B., Mandhane, P. J., Turvey, S., Moraes, T. J., Sears, M. R., Subbarao, P., Sycuro, L. K., Azad, M. B., & Arrieta, M.-C. (2020). Consumption of artificially sweetened beverages during pregnancy impacts infant gut microbiota and body mass index. *BioRxiv*. DOI: 10.1101/2020.04.20.050195
67. Lauder, A. P., Roche, A. M., Sherrill-Mix, S., Bailey, A., Laughlin, A. L., Bittinger, K., Leite, R., Elovitz, M. A., Parry, S., & Bushman, F. D. (2016). Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. *Microbiome*, 4(1), 29. DOI: 10.1186/s40168-016-0172-3
68. Laue, H. E., Brennan, K. J., Gillet, V., Abdelouahab, N., Coull, B. A., Weisskopf, M. G., Burris, H. H., Zhang, W., Takser, L., & Baccarelli, A. A. (2019). Associations of prenatal exposure to polybrominated diphenyl ethers and polychlorinated biphenyls with long-term gut microbiome structure: A pilot study. *Environmental epidemiology (Philadelphia, Pa.)*, 3(1). DOI: 10.1097/EE9.0000000000000039
69. Laue, H. E., Moroishi, Y., Jackson, B. P., Palys, T. J., Madan, J. C., & Karagas, M. R. (2020). Nutrient-toxic element mixtures and the early postnatal gut microbiome and in a United States longitudinal birth cohort. *Environment International*, 138, 105613. DOI: 10.1016/j.envint.2020.105613
70. Li, B., Ding, Y., Cheng, X., Sheng, D., Xu, Z., Rong, Q., Wu, Y., Zhao, H., Ji, X., & Zhang, Y. (2020). Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. *Chemosphere*, 244, 125492. DOI: 10.1016/j.scitotenv.2018.03.051
71. Lu, L., Luo, T., Zhao, Y., Cai, C., Fu, Z., & Jin, Y. (2019). Interaction between microplastics and microorganism as well as gut microbiota: A consideration on environmental animal and human health. *Science of the Total Environment*, 667, 94-100. DOI: 10.1016/j.scitotenv.2019.02.380
72. Luo, T., Zhang, Y., Wang, C., Wang, X., Zhou, J., Shen, M., Zhao, Y., Fu, Z., & Jin, Y. (2019). Maternal exposure to different sizes of polystyrene microplastics during gestation causes metabolic disorders in their offspring. *Environmental Pollution*, 255, 113122. DOI: 10.1016/j.envpol.2019.113122

73. Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., & Balamurugan, R. (2020). The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? *Nutrients*, 12(5), 1474. DOI: 10.3390/nu12051474
74. Malik, V. S., Li, Y., Pan, A., De Koning, L., Schernhammer, E., Willett, W. C., & Hu, F. B. (2019). Long-Term Consumption of Sugar-Sweetened and Artificially Sweetened Beverages and Risk of Mortality in US Adults. *Circulation*, 139(18), 2113-2125. DOI: 10.1161/CIRCULATIONAHA.118.037401
75. Mapesa, J. O., Maxwell, A. L., & Ryan, E. P. (2016). An exposome perspective on environmental enteric dysfunction. *Environmental health perspectives*, 124(8), 1121-1126. DOI: 10.1289/ehp.1510459
76. Marchesi, J.R., Ravel, J. The vocabulary of microbiome research: a proposal. *Microbiome* 3, 31 (2015). DOI: 10.1186/s40168-015-0094-5
77. Margina, D., Nițulescu, G. M., Ungurianu, A., Mesnage, R., Goumenou, M., Sarigiannis, D. A., Aschner, M., Spandidos, D. A., Renieri, E. A., & Hernández, A. F. (2019). Overview of the effects of chemical mixtures with endocrine disrupting activity in the context of real-life risk simulation (RLRS): An integrative approach. *World Academy of Sciences Journal*, 1(4), 157-164. DOI: 10.3892/wasj.2019.17
78. Maslova, E., Strøm, M., Olsen, S. F., & Halldorsson, T. I. (2013). Consumption of Artificially-Sweetened Soft Drinks in Pregnancy and Risk of Child Asthma and Allergic Rhinitis. *PLoS ONE*, 8(2), e57261. DOI: 10.1371/journal.pone.0057261
79. Mason, S. A., Welch, V. G., & Neratko, J. (2018). Synthetic polymer contamination in bottled water. *Frontiers in chemistry*, 6, 407. DOI: 10.3389/fchem.2018.00407
80. Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., Belzer, C., Palacio, S. D., Montes, S. A., & Mancabelli, L. (2017). The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews*, 81(4). DOI: 10.1128/MMBR.00036-17
81. Mueller, N. T., Bakacs, E., Combellick, J., Grigoryan, Z., & Dominguez-Bello, M. G. (2015). The infant microbiome development: Mom matters. *Trends in molecular medicine*, 21(2), 109-117. DOI: 10.1016/j.molmed.2014.12.002
82. Murphy, E. F., Cotter, P. D., Healy, S., Marques, T. M., O'Sullivan, O., Fouhy, F., Clarke, S. F., O'Toole, P. W., Quigley, E. M., Stanton, C., Ross, P. R., O'Doherty, R. M., & Shanahan, F. (2010). Composition and energy harvesting capacity of the gut microbiota: Relationship to diet, obesity and time in mouse models. *Gut*, 59(12), 1635-1642. DOI: 10.1136/gut.2010.215665
83. Murphy, V. E., Smith, R., Giles, W. B., & Clifton, V. L. (2006). Endocrine regulation of human fetal growth: The role of the mother, placenta, and fetus. *Endocrine reviews*, 27(2), 141-169. DOI: 10.1210/er.2005-0011
84. Mustieles, V., Zhang, Y., Yland, J., Braun, J. M., Williams, P. L., Wylie, B. J., Attaman, J. A., Ford, J. B., Azevedo, A., & Calafat, A. M. (2020). Maternal and paternal preconception exposure to phenols and preterm birth. *Environment International*, 137, 105523. DOI: 10.1016/j.envint.2020.105523
85. Naidu, K. S. B. (2020). Engineered nanoparticles: Hazards and Risk Assessment upon Exposure-A Review. *Current Trends in Biotechnology & Pharmacy*, 14(1). DOI: 10.5530/ctbp.2020.1.11
86. Nejrup, R. G., Licht, T. R., & Hellgren, L. I. (2017). Fatty acid composition and phospholipid types used in infant formulas modifies the establishment of human gut bacteria in germ-free mice. *Scientific reports*, 7(1), 1-11. DOI: 10.1038/s41598-017-04298-0
87. Ogra, P. L., Walker, W. A., & Lönnerdal, B. (2020). Milk, Mucosal Immunity and the Microbiome: Impact on the Neonate (Vol. 94). Karger. DOI: 10.1159/isbn.978-3-318-06685-2

88. Organization, W. H. (2006). Principles for evaluating health risks in children associated with exposure to chemicals. World Health Organization.
89. Pajewska-Szmyt, M., Sinkiewicz-Darol, E., & Gadzała-Kopciuch, R. (2019). The impact of environmental pollution on the quality of mother's milk. *Environmental Science and Pollution Research*, 26(8), 7405-7427. DOI: 10.1007/s11356-019-04141-1
90. Pannaraj, P. S., Li, F., Cerini, C., Bender, J. M., Yang, S., Rollie, A., Adisetiyo, H., Zabih, S., Lincez, P. J., & Bittinger, K. (2017). Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA pediatrics*, 171(7), 647-654. DOI: 10.1001/jamapediatrics.2017.0378
91. Park, Y., Lee, A., Choi, K., Kim, H.-J., Lee, J. J., Choi, G., Kim, S., Kim, S. Y., Cho, G. J., & Suh, E. (2018). Exposure to lead and mercury through breastfeeding during the first month of life: A CHECK cohort study. *Science of The Total Environment*, 612, 876-883. DOI: 10.1016/j.scitotenv.2017.08.079
92. Perlroth, N. H., & Branco, C. W. C. (2017). Current knowledge of environmental exposure in children during the sensitive developmental periods. *Jornal de pediatria*, 93(1), 17-27. DOI: 10.1016/j.jpmed.2016.07.002
93. Pietroiusti, A., Magrini, A., & Campagnolo, L. (2016). New frontiers in nanotoxicology: Gut microbiota/microbiome-mediated effects of engineered nanomaterials. *Toxicology and applied pharmacology*, 299, 90-95. DOI: 10.1016/j.taap.2015.12.017
94. Pinget, G., Tan, J., Janac, B., Kaakoush, N. O., Angelatos, A. S., O'Sullivan, J., Koay, Y. C., Sierro, F., Davis, J., & Divakarla, S. K. (2019). Impact of the food additive titanium dioxide (E171) on gut microbiota-host interaction. *Frontiers in nutrition*, 6, 57. DOI: 10.3389/fnut.2019.00057
95. Porpora, M. G., Lucchini, R., Abballe, A., Ingelido, A. M., Valentini, S., Fuggetta, E., Cardi, V., Ticino, A., Marra, V., & Fulgenzi, A. R. (2013). Placental transfer of persistent organic pollutants: A preliminary study on mother-newborn pairs. *International journal of environmental research and public health*, 10(2), 699-711. DOI: 10.3390/ijerph10020699
96. Rackaityte, E., Halkias, J., Fukui, E. M., Mendoza, V. F., Hayzelden, C., Crawford, E. D., Fujimura, K. E., Burt, T. D., & Lynch, S. V. (2020). Viable bacterial colonization is highly limited in the human intestine in utero. *Nature Medicine*, 26(4), 599-607. DOI: 10.1038/s41591-020-0761-3
97. Rager, J. E., Bangma, J., Carberry, C., Chao, A., Grossman, J., Lu, K., Manuck, T. A., Sobus, J. R., Szilagyi, J., & Fry, R. C. (2020). Review of the environmental prenatal exposome and its relationship to maternal and fetal health. *Reproductive Toxicology*. DOI: 10.1016/j.reprotox.2020.02.004
98. Rainieri, S., & Barranco, A. (2019). Microplastics, a food safety issue? *Trends in food science & technology*, 84, 55-57. DOI: 10.1016/j.tifs.2018.12.009
99. Reddivari, L., Veeramachaneni, D. N. R., Walters, W. A., Lozupone, C., Palmer, J., Hewage, M. K. K., Bhatnagar, R., Amir, A., Kennett, M. J., Knight, R., & Vanamala, J. K. P. (2017). Perinatal Bisphenol A Exposure Induces Chronic Inflammation in Rabbit Offspring via Modulation of Gut Bacteria and Their Metabolites. *MSystems*, 2(5), e00093-17. PubMed. DOI: 10.1128/mSystems.00093-17
100. Renz, H., Holt, P. G., Inouye, M., Logan, A. C., Prescott, S. L., & Sly, P. D. (2017). An exposome perspective: Early-life events and immune development in a changing world. *Journal of Allergy and Clinical Immunology*, 140(1), 24-40. DOI: 10.1016/j.jaci.2017.05.015
101. Robertson, R. C., Manges, A. R., Finlay, B. B., & Prendergast, A. J. (2019). The human microbiome and child growth—first 1000 days and beyond. *Trends in microbiology*, 27(2), 131–147. DOI: 10.1016/j.tim.2018.09.008
102. Rosenfeld, C. S. (2017). Gut Dysbiosis in Animals Due to Environmental Chemical Exposures. *Frontiers in Cellular and Infection Microbiology*, 7, 396. DOI: 10.3389/fcimb.2017.00396

103. Rothenberg, S. E., Keiser, S., Ajami, N. J., Wong, M. C., Gesell, J., Petrosino, J. F., & Johs, A. (2016). The role of gut microbiota in fetal methylmercury exposure: Insights from a pilot study. *Toxicology letters*, 242, 60-67. DOI: 10.1016/j.toxlet.2015.11.022
104. Rothenberg, S. E., Wagner, C. L., Alekseyenko, A. V., & Azcarate-Peril, M. A. (2019). Longitudinal changes during pregnancy in gut microbiota and methylmercury biomarkers, and reversal of microbe-exposure correlations. *Environmental research*, 172, 700-712. DOI: 10.1016/j.envres.2019.01.014
105. Rother, K. I., Sylvestsky, A. C., Walter, P. J., Garraffo, H. M., & Fields, D. A. (2018). Pharmacokinetics of Sucralose and Acesulfame-Potassium in Breast Milk Following Ingestion of Diet Soda: *Journal of Pediatric Gastroenterology and Nutrition*, 66(3), 466-470. DOI: 10.1097/MPG.0000000000001817
106. Ruiz-Ojeda, F. J., Plaza-Díaz, J., Sáez-Lara, M. J., & Gil, A. (2019). Effects of sweeteners on the gut microbiota: A review of experimental studies and clinical trials. *Advances in Nutrition*, 10(suppl_1), S31-S48. DOI: 10.1093/advances/nmy037
107. Sabbioni, G., Berset, J.-D., & Day, B. W. (2020). Is It Realistic to Propose Determination of a Lifetime Internal Exposome? *Chemical Research in Toxicology*, 33(8), 2010-2021. DOI: 10.1021/acs.chemrestox.0c00092
108. Sbihi, H., Boutin, R. C., Cutler, C., Suen, M., Finlay, B. B., & Turvey, S. E. (2019). Thinking bigger: How early-life environmental exposures shape the gut microbiome and influence the development of asthma and allergic disease. *Allergy*, 74(11), 2103-2115. DOI: 10.1111/all.13812
109. Schiffman, S. S., & Nagle, H. T. (2019). Revisited: Assessing the in vivo data on low/no-calorie sweeteners and the gut microbiota. *Food and Chemical Toxicology*, 132, 110692. DOI: 10.1016/j.fct.2019.110692
110. Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T., & Liebmann, B. (2019). Detection of various microplastics in human stool: A prospective case series. *Annals of internal medicine*, 171(7), 453-457. DOI: 10.7326/M19-0618
111. Simon, J.-C., Marchesi, J. R., Mougel, C., & Selosse, M.-A. (2019). Host-microbiota interactions: From holobiont theory to analysis. *Microbiome*, 7(1), 1-5. DOI: 10.1186/s40168-019-0619-4
112. Sitarik, A. R., Arora, M., Austin, C., Bielak, L. F., Eggers, S., Johnson, C. C., Lynch, S. V., Kyun Park, S., Hank Wu, K.-H., Yong, G. J. M., & Cassidy-Bushrow, A. E. (2020). Fetal and early postnatal lead exposure measured in teeth associates with infant gut microbiota. *Environment International*, 144, 106062. DOI: 10.1016/j.envint.2020.106062
113. Sprockett, D., Fukami, T., & Relman, D. A. (2018). Role of priority effects in the early-life assembly of the gut microbiota. *Nature Reviews Gastroenterology & Hepatology*, 15(4), 197-205. DOI: 10.1038/nrgastro.2017.173
114. Stichelen, O.-V., Rother, K. I., & Hanover, J. A. (2019). Maternal exposure to non-nutritive sweeteners impacts progeny's metabolism and microbiome. *Frontiers in microbiology*, 10, 1360. DOI: 10.3389/fmicb.2019.01360
115. Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaïss, C. A., Maza, O., Israeli, D., Zmora, N., Gilad, S., Weinberger, A., Kuperman, Y., Harmelin, A., Kolodkin-Gal, I., Shapiro, H., Halpern, Z., Segal, E., & Elinav, E. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*, 514(7521), 181-186. DOI: 10.1038/nature13793
116. Sylvestsky, A. C., Gardner, A. L., Bauman, V., Blau, J. E., Garraffo, H. M., Walter, P. J., & Rother, K. I. (2015). Nonnutritive sweeteners in breast milk. *Journal of Toxicology and Environmental Health, Part A*, 78(16), 1029-1032. DOI: 10.1080/15287394.2015.1053646
117. Tang, M., Xu, C., Chen, K., Yan, Q., Mao, W., Liu, W., & Ritz, B. (2019). Hexachlorocyclohexane exposure alters the microbiome of colostrum in Chinese

- breastfeeding mothers. *Environmental Pollution*, 254, 112900. DOI: 10.1016/j.envpol.2019.07.068
118. Teng, C., Jia, J., Wang, Z., Sharma, V. K., & Yan, B. (2019). Size-dependent maternal-fetal transfer and fetal developmental toxicity of ZnO nanoparticles after oral exposures in pregnant mice. *Ecotoxicology and environmental safety*, 182, 109439. DOI: 10.1016/j.ecoenv.2019.109439
119. Teng, C., Jia, J., Wang, Z., & Yan, B. (2020). Oral Co-Exposures to zinc oxide nanoparticles and CdCl₂ induced maternal-fetal pollutant transfer and embryotoxicity by damaging placental barriers. *Ecotoxicology and Environmental Safety*, 189, 109956. DOI: 10.1016/j.ecoenv.2019.109956
120. Theis, K. R., Romero, R., Winters, A. D., Greenberg, J. M., Gomez-Lopez, N., Alhousseini, A., Bieda, J., Maymon, E., Pacora, P., & Fettweis, J. M. (2019). Does the human placenta delivered at term have a microbiota? Results of cultivation, quantitative real-time PCR, 16S rRNA gene sequencing, and metagenomics. *American journal of obstetrics and gynecology*, 220(3), 267. e1-267. e39. DOI: 10.1016/j.ajog.2018.10.018
121. Thompson, L. A., & Darwish, W. S. (2019). Environmental chemical contaminants in food: Review of a global problem. *Journal of toxicology*, 2019. DOI: 10.1155/2019/2345283
122. Timmerman, H. M., Rutten, N. B., Boekhorst, J., Saulnier, D. M., Kortman, G. A., Contractor, N., Kullen, M., Floris, E., Harmsen, H. J., & Vlieger, A. M. (2017). Intestinal colonisation patterns in breastfed and formula-fed infants during the first 12 weeks of life reveal sequential microbiota signatures. *Scientific reports*, 7(1), 1-10. DOI: 10.1038/s41598-017-08268-4
123. Tojo, R. (2014). Intestinal microbiota in health and disease: Role of bifidobacteria in gut homeostasis. *World Journal of Gastroenterology*, 20(41), 15163. DOI: 10.3748/wjg.v20.i41.15163
124. Tsatsakis, A. (2019). The concept of RLRS for toxicology safety evaluations in our modern world. *TOXICOLOGY LETTERS*, 314, S40-S40. ISSN 0378-4274
125. Tsatsakis, Am, Kouretas, D., Tzatzarakis, M., Stivaktakis, P., Tsarouhas, K., Golokhvast, K., Rakitskii, V., Tutelyan, V., Hernandez, A., Rezaee, R., Chung, G., Fenga, C., Engin, A., Neagu, M., Arsene, A., Docea, A., Gofita, E., Calina, D., Taitzoglou, I., ... Tsitsimpikou, C. (2017). Simulating real-life exposures to uncover possible risks to human health: A proposed consensus for a novel methodological approach. *Human & Experimental Toxicology*, 36(6), 554-564. DOI: 10.1177/0960327116681652
126. Tsatsakis, Aristidis, Docea, A. O., Constantin, C., Calina, D., Zlatian, O., Nikolouzakakis, T. K., Stivaktakis, P. D., Kalogeraki, A., Liesivuori, J., & Tzanakakis, G. (2019). Genotoxic, cytotoxic, and cytopathological effects in rats exposed for 18 months to a mixture of 13 chemicals in doses below NOAEL levels. *Toxicology Letters*, 316, 154-170. DOI: 10.1016/j.toxlet.2019.09.004
127. Tsiaoussis, J., Antoniou, M. N., Koliarakis, I., Mesnage, R., Vardavas, C. I., Izotov, B. N., Psaroulaki, A., & Tsatsakis, A. (2019). Effects of single and combined toxic exposures on the gut microbiome: Current knowledge and future directions. *Toxicology Letters*, 312, 72-97. DOI: 10.1016/j.toxlet.2019.04.014
128. Uranga, C. C., Arroyo, P., Duggan, B. M., Gerwick, W. H., & Edlund, A. (2020). Commensal Oral *Rothia mucilaginosa* Produces Enterobactin, a Metal-Chelating Siderophore. *MSystems*, 5(2), e00161-20, /msystems/5/2/msys.00161-20.atom. DOI: 10.1128/mSystems.00161-20
129. Van Genderen, E., Adams, W., Dwyer, R., Garman, E., & Gorsuch, J. (2015). Modeling and interpreting biological effects of mixtures in the environment: Introduction to the metal mixture modeling evaluation project. *Environmental Toxicology and Chemistry*, 34(4), 721-725. DOI: 10.1002/etc.2750

130. Vatanen, T., Kostic, A. D., d’Hennezel, E., Siljander, H., Franzosa, E. A., Yassour, M., Kolde, R., Vlamakis, H., Arthur, T. D., Hämäläinen, A.-M., Peet, A., Tillmann, V., Uibo, R., Mokurov, S., Dorshakova, N., Ilonen, J., Virtanen, S. M., Szabo, S. J., Porter, J. A., ... Xavier, R. J. (2016). Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. *Cell*, 165(4), 842-853. DOI: 10.1016/j.cell.2016.04.007
131. Victora, C. G., Bahl, R., Barros, A. J., França, G. V., Horton, S., Krasevec, J., Murch, S., Sankar, M. J., Walker, N., & Rollins, N. C. (2016). Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. *The Lancet*, 387(10017), 475-490. DOI: 10.1016/S0140-6736(15)01024-7
132. Viennois, E., Merlin, D., Gewirtz, A. T., & Chassaing, B. (2017). Dietary emulsifier-induced low-grade inflammation promotes colon carcinogenesis. *Cancer research*, 77(1), 27-40. DOI: 10.1158/0008-5472
133. Wang, S., Ryan, C. A., Boyaval, P., Dempsey, E. M., Ross, R. P., & Stanton, C. (2020). Maternal vertical transmission affecting early-life microbiota development. *Trends in microbiology*, 28(1), 28-45. DOI: 10.1016/j.tim.2019.07.010
134. WHO. (2006). Principles for evaluating health risks in children associated with exposure to chemicals.
135. Wu, J., Wen, X. W., Faulk, C., Boehnke, K., Zhang, H., Dolinoy, D. C., & Xi, C. (2016). Perinatal lead exposure alters gut microbiota composition and results in sex-specific bodyweight increases in adult mice. *Toxicological Sciences*, 151(2), 324-333. DOI: doi.org/10.1093/toxsci/kfw046
136. Yang, Y.-N., Yang, Y.-C. S., Lin, I.-H., Chen, Y.-Y., Lin, H.-Y., Wu, C.-Y., Su, Y.-T., Yang, Y.-J., Yang, S.-N., & Suen, J.-L. (2019). Phthalate exposure alters gut microbiota composition and IgM vaccine response in human newborns. *Food and Chemical Toxicology*, 132, 110700. DOI: 10.1016/j.fct.2019.110700
137. Zhang, G.-H., Chen, M.-L., Liu, S.-S., Zhan, Y.-H., Quan, Y., Qin, Y.-M., & Deng, S.-P. (2011). Effects of mother’s dietary exposure to acesulfame-K in pregnancy or lactation on the adult offspring’s sweet preference. *Chemical senses*, 36(9), 763-770. DOI: 10.1093/chemse/bjr050
138. Zhang, T., Li, Q., Cheng, L., Buch, H., & Zhang, F. (2019). *Akkermansia muciniphila* is a promising probiotic. *Microbial Biotechnology*, 12(6), 1109-1125. DOI: 10.1111/1751-7915.13410
139. Zhu, Y., Lai, J., Liao, X., & Liu, S. (2018). Screening of lactic acid bacteria strains for their ability to bind phthalate monoesters in vitro and the binding characteristics. *Food Control*, 90, 364-371. DOI: 10.1016/j.foodcont.2018.02.013
140. Zhuang, L., Chen, H., Zhang, S., Zhuang, J., Li, Q., & Feng, Z. (2019). Intestinal microbiota in early life and its implications on childhood health. *Genomics, proteomics & bioinformatics*, 17(1), 13-25. DOI: 10.1016/j.gpb.2018.10.002