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Sandys, Oliver; te Velde, Anje

Published in:
Digestive diseases and sciences

DOI:
10.1007/s10620-021-07327-1

Published: 01/09/2022

Document Version
Peer reviewed version

Citation for pulished version (APA):
Sandys, O., & te Velde, A. (2022). Raising the Alarm: Environmental Factors in the Onset and Maintenance of Chronic (Low-Grade) Inflammation in the Gastrointestinal Tract. *Digestive diseases and sciences*, *67*(9), 4355-4368. https://doi.org/10.1007/s10620-021-07327-1

DOI : 10.1007/s10620-021-07327-1

Raising the alarm: environmental factors in the onset and maintenance of chronic (low-grade) inflammation in the gastrointestinal tract

Oliver Sandys[1,2], Anje te Velde[1]

Affiliations:

(1) AmsterdamUMC, University of Amsterdam, Tytgat Institute for Liver and Intestinal Research, AGEM, Amsterdam, the Netherlands.

(2) Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

Abstract

Chronic inflammatory disease of the gastrointestinal (GI) tract is defined by several pathophysiological characteristics, such as dysbiosis of the microbiota, epithelial barrier hyperpermeability, systemic dissemination of endotoxins and chronic inflammation. In addition to well reported environmental factors in non-communicable disease, such as smoking, diet, and exercise, humans are frequently exposed to myriads more environmental factors, from pesticides to food additives. Such factors are ubiquitous across both our diet and indoor/outdoor environments. A major route of human exposure to these factors is ingestion, which frequently occurs due to their intentional *addition* (intentional food additives) and/or unintentional *contamination* (unintentional food contaminants) of food products – often linked to environmental pollution. Understanding how this persistent, diverse exposure impacts GI health is of paramount importance, as deterioration of the GI barrier is proposed to be the first step towards systemic inflammation and chronic disease. Therefore, we aim to evaluate the impact of ingestion of environmental factors on inflammatory processes in the GI tract. In this review we highlight human exposure to intentional food additives (e.g. emulsifiers, bulking agents) and unintentional food contaminants (e.g. persistent organic pollutants, pesticides, microplastics), then present evidence for their association with chronic disease, modification of the GI microbiota, increased permeability of the GI barrier, systemic dissemination of endotoxins, local (and distal) pro-inflammatory signalling, and induction of oxidative stress and/or endoplasmic reticulum stress. We also propose a link to NLRP3-inflammasome activation. These findings highlight the contribution of common environmental factors towards deterioration of GI

health and the induction of pathophysiology associated with onset and maintenance of chronic inflammation in the GI tract.

Keywords: Chronic gastrointestinal inflammation, Environmental factors, Food additives, Food contaminants, NLRP3 inflammasome, Regulatory questions

Modern civilization presents the immune system new challenges

Throughout the course of human evolution we have developed an intimate relationship with the complex system of the natural world; with the air we breathe into our lungs, the food we ingest and digest in our intestines, and through contact with our skin. In constant surveillance of this relationship is the immune system, which strives to maintain homeostasis in response to tissue damage, infection or introduction of other foreign agents that are exposed to us through our surrounding ecosystem [1]. The natural release of toxic substances into our environment is usually within the boundaries of ecological equilibrium, to which the human body can respond appropriately within homeostatic equilibrium, and without disease [2]. Human exposure to exogenous substances – through ingestion, inhalation, or dermal absorption – requires a bodily response of biotransformation, conjugation, isolation, and/or excretion, which can all serve to maintain the body's homeostasis and prevent disease [3,4]. However, a paradigm shift in both diet and ecology emerged with the dawn of the 19th century industrial revolution. Due to the boom in (naturally derived and artificial) chemical production, humans – and indeed all other species – are now exposed to a massive array of potential toxicants, at a scale unlike anything we have faced before [5]. Over the past half century alone, >150,000 synthetic chemicals have been created, with an estimated 2,000 new chemicals being produced annually for a huge range of agricultural and industrial applications

[6]. Consequently, the variety, nature, and pervasiveness of our exposome – all the human environmental exposures (non-genetic) starting from the prenatal period – has drastically increased [7].

The changes to our exposome combined with rapid modernisation (and indeed, urbanisation), have led to drastic modification of our human ecology – diet, physical activity, microbial diversity, and population density – away from the previously defining natural parameters [8]. Today in many parts of the world the human population spends up to 90% of their lives indoors; in isolation, in contrast to our past co-evolution within microbially diverse ecosystems [9,10]. Hygiene, a novel outcome of modernisation, has led to a subsequent reduction of infectious stimuli against which our genes have been selected [8]. Together, these novel scenarios present our immune system new challenges (Figure 1). Perhaps the most critical interface at which the human body must adapt to these new challenges is the GI tract, where the epithelial barrier forms the largest external surface area of the body [11]. Thus, it is of the upmost importance to understand how ingested environmental factors impact on GI health and immune homeostasis – the tightly regulated balance of pro- and anti-inflammatory mechanisms that protect against undesired inflammatory activity to harmless antigens of the environment, microbiota or diet [12,13].

SCOPE: In this review we evaluate the impact of pervasive environmental factors that form part of the human exposome. Environmental factors are defined herein as, "*exogenous substances that arise through human production that are ingested due to contamination and/or modification of the environment, food products, and/or drinking water"*. We first highlight a (non-exhaustive) range of pervasive environmental factors that constitute our ingestible exposome, before presenting their detrimental effects on GI health.

This paper hypothesises that such environmental factors cause a sustained, life-long perturbation of immune homeostasis in the gastrointestinal tract, contributing to the onset and maintenance of chronic inflammation.

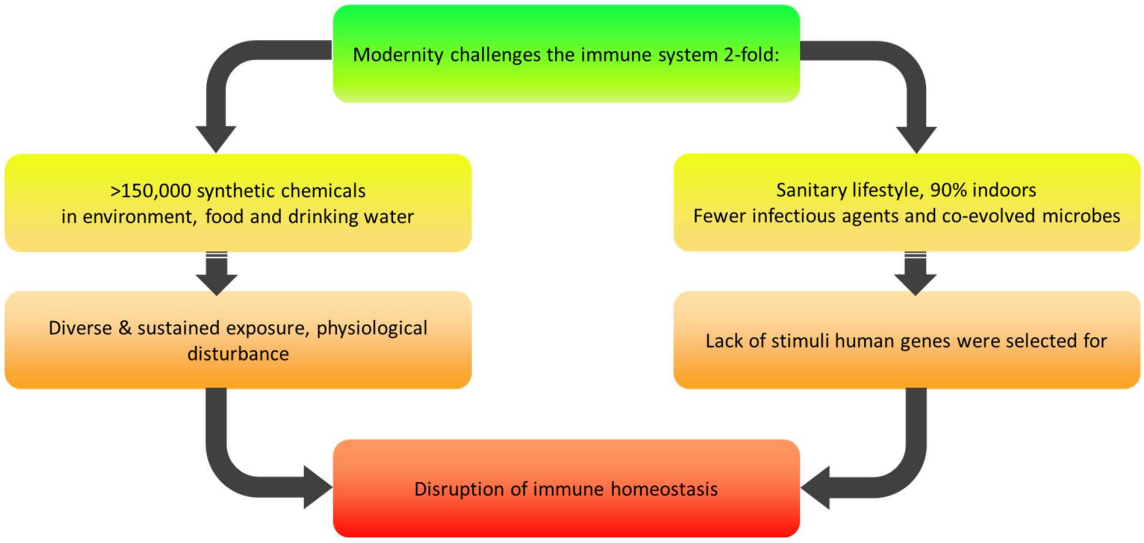

Figure 1 – Modern civilization generates novel challenges to the immune system. Co-evolution with certain microbes prevalent in natural ecosystems provided selective pressure for human genes. However, with today's modern lifestyle, the human population spends up to 90% of their lives indoors. This new sterile living environment and associated deficiency in infectious stimuli, together with excessive chemical exposure and physiological disturbances, leads to disruption of immune homeostasis.

Human exposure to ingested environmental factors

A major route of human exposure to environmental factors is through the ingestion of contaminated (i.e. pollutants, also airborne) or modified (i.e. additives) food and drinking water [14]. Consequentially, ingestion of chemicals added intentionally or unintentionally to

food exposes the GI tract to an extraordinary range of compounds (Figure 2). Moreover, the resident GI microbiota – which can interact with ingested compounds – continue to emerge as a major factor in the etiology of chronic inflammation, further emphasizing the need to understand the effects of ingested environmental factors [15]. Considering the pathophysiological potential of local – and systemic – immune-dysregulation originating in the GI tract, this review focuses exclusively on ingested environmental factors. In the following section human exposure to such factors is introduced to further establish the relevance of investigating their role in chronic GI inflammation. Classifications are made to describe both intentional food additives (IFAs), widely used in the food and drink industry, and unintentional food contaminants (UFCs), such as pesticides and persistent organic pollutants (POPs) that are frequently detected in our food and drinking water [16,17]. Despite the relevance of particulates and heavy metals as ingestible environmental factors, they are not given further attention in this review, although both are recognized for their relevance as toxicants with the potential to contribute to inflammatory disease [3,18,19].

Intentional Food Additives (IFAs)

Of the two classifications made in this review, IFAs are the overt environmental factors, clearly labelled on food and drink packaging. Over 2500 different food additives are added to food to perform specific functions, such as to enhance taste, preserve, or stabilize [20]. Approval of a food additive after safety testing results in them being given an E number ('E' stands for 'Europe'). A study in French supermarkets found 54% of 126,000 food products contained an additive, with 11% containing at least five [21]. Ultra-processed foods often contain mixtures of additives and are increasingly consumed in low and middle income countries, while accounting for half of calorie intake in high income countries [22]. Marino et

al. recently provided a comprehensive review of the level of ultra-processed food consumption across 21 countries and 100 studies [23].

Many food additives exposed to the GI tract are micro- or nano-particles, such as titanium dioxide (named E171 in Europe), used for whitening and brightening foods like confectionary, white sauces, and particular powdered foods; and silicates/aluminosilicates (E554, E556 and E559), which are used as anti-caking agents, and also in cheeses, sugars and milk powder [24]. Dietary intake of inorganic microparticles in the UK amounts to 40mg/person/day, or $10^{12\text{-}14}$ particles per person [24]. As ingested compounds, food additives including micro- or nano-particles, pass through the GI tract and GI microbiota. This presents an intriguing mechanism by which they may contribute to dysregulation of the intricate immune-GI mucosa-GI microbiota interactions, and induce an inflammatory response [25].

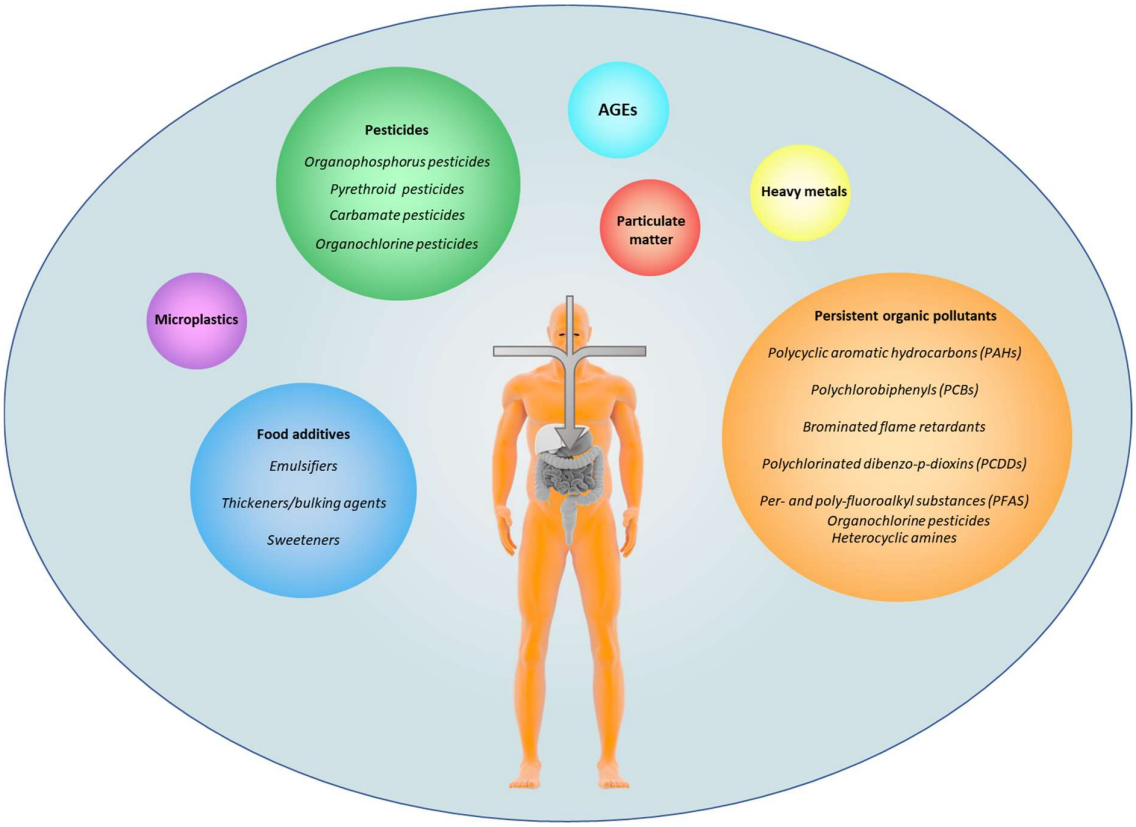

Figure 2 – An overview of some frequently ingested classes of environmental factors, including intentional food and drink additives, such as emulsifiers, thickening/bulking agents, or sweeteners, and unintentional food and drink contaminants, such as persistent organic pollutants, pesticides, heavy metals, microplastics, particulates and advanced glycation end-products (AGEs).

Unintentional Food Contaminants (UFCs)

A much bigger catalogue of environmental factors in chronic inflammatory disease is of substances that are ingested unknowingly, through environmental exposure, or contaminated food and drinking water, such as POPs, pesticides, heavy metals, microplastics, particulates and advanced glycation end-products (AGEs).

Persistent organic pollutants (POPs)

POPs, also known as the "forever chemicals" due to their extreme persistence and resistance to degradation, are emitted from industry, along with being heavily applied to plant protection products [26]. Many synthetic POPs contaminate food, such as polycyclic aromatic hydrocarbons, polychlorobiphenyls, brominated flame retardants, polychlorinated dibenzo-p-dioxins, per- and polyfluoroalkyl substances (PFAS), and many pesticides [13]. POPs readily bioaccumulate in human fat tissues, allowing them to persist for decades in the human body [27]. Moreover, their pervasiveness across environment, food, and water means human exposure and bioaccumulation of POPs is relatively common; a 2014 study by the Netherland's National Institute for Public Health and Environment (RIVM) found many POPs in breast milk, including several PFAS – known immunotoxins – and nine of the twelve so-called 'Dirty Dozen' chemicals (a group of POPs identified in 2001) [26,28]. With >4700 manufactured, PFAS represent a major class of POP that humans are exposed to via drinking water, food and indoor/outdoor environments, and which are readily absorbed through the mammalian gastrointestinal tract [29,30]. Concentrations of PFAS and/or perfluorooctane sulfonates are above Environmental Protection Agency (EPA) guidelines in drinking water supplies serving over six million Americans, while the European Food Safety Authority (EFSA) concluded a considerable proportion of the European population faced exposure above the tolerable weekly intake [30,31]. Although use of "Legacy PFAS" has declined in use due to widespread bans or heavy restrictions, replacement PFAS are readily available despite insufficient research on toxicity [28].

Pesticides

Pesticides make up one of the most common environmental toxins, with around 2 million tons applied per year worldwide [32]. One landmark study estimated that just 0.3% of pesticides applied reach the target organism, while the other 99.7% disperses into the surrounding ecosystem [33]. Human exposure to pesticides arises from environmental sources, such as surface water, drinking water, and food – importantly, pesticides can accumulate in leaves, grains and fruit, which prevents removal of residues by washing [34]. Organophosphorus pesticides are the most heavily used class of herbicides worldwide and frequent UFCs, for example, glyphosate exposure occurs via contaminated drinking water, precipitation, air, and food – including the global soybean supply [16]. A recent study in Germany tested 399 adult urine samples and detected glyphosate residues above detection limits in 32%, while the glyphosate metabolite aminomethylphosphonic acid was detected in 40% of samples [35]. Furthermore, another prevalent organophosphorus pesticide, chlorpyrifos, has been frequently detected in up to 38.3% of food tested at levels that have exceeded the EFSA acute reference dose for food products [36].

Many pesticides are also considered POPs, for example organochlorine pesticides, such as dichlorodiphenyltrichloroethanes (DDTs), which have half-lives of 2-15 years; increasing the potential for bioaccumulation in the human body [37,38]. A recent study in South Africa found that several organochlorine pesticides, such as hexachlorobenzene and multiple DDTs, occur in breast milk at levels above WHO limits [39]. Moreover, a study conducted by the RIVM in the Netherlands also found many organochlorine pesticides – including DDT and hexachlorobenzene – in breast milk [26].

Microplastics

Further to contaminators such as POPs and pesticides, exposure to contaminating microparticles is also common. Human exposure occurs via inhalation, dermal absorption, and the major route, ingestion, which alone is estimated to amount to 39,000-52,000 particles person $^{-1}$ year^{-1}, based on consumption of food stuff [40]. In the GI tract cellular uptake of micro- or nanoplastics occurs via multiple mechanisms, including via M cells of the Peyer's patches, endocytosis, and persorption [41]. This may include microplastics, heavy metals and particulates. Common routes of exposure include mussels, commercial fish, table salt, sugar, (bottled) water, fruits and vegetables, teabags and household dust released from plastic components of carpets, curtains and other household items [41–44].

Microplastics consist of more than plastic alone. They can harbour a range of endogenous POPs that rapidly desorb under GI physiological conditions (38 °C, pH 4) [45]. In addition, plastics contain reactive oxygen species (ROS), which can increase in concentration after interaction with light, or presence of transition metals that adsorb to the microplastic surface [41,45]. Microplastics also function as vectors: agglomerations of biomolecule conjugates can form on their surface, consisting of intestinal endoprotein, endotoxins, whole and partially digested bacteria and non-absorbed food antigens; while the hydrophobic nature of microplastics drives adsorption of POPs, including polycyclic aromatic hydrocarbons, polychlorobiphenyls, per- and polyfluoroalkyl substances and organochlorine pesticides [42]. In addition, microplastics can also accumulate heavy metals. Their capacity as vectors for microorganisms is also proposed to assist in transport of pathogens across the GI barrier, as the surface of microplastics is rapidly colonised leading to establishment of protective biofilms[46].

Advanced glycation end-products

Lastly, advanced glycation end-products (AGEs) are recognised as dietary UFCs. AGEs are produced due to protein or lipid modification under certain conditions, such as rapid heating or high sugar concentrations, through non-enzymatic glycation and oxidation reactions between reducing sugars and proteins, and lipids and DNA [47]. Endogenous AGE formation is usually a slow process, but it can be sped up under conditions of oxidative stress [48]. Exogenous AGEs are ingested via our diet, especially in food with high fat or protein content that are cooked at high temperatures, particularly in dry heat – foods that are abundant in the western diet [49]. Of all ingested AGEs, approximately 10% are absorbed through the intestine, with around one third then excreted in the urine [50].

Environmental factors are associated with GI dysbiosis, increased GI barrier permeability, and GI oxidative stress and ER stress

Today, the World Health Organization (WHO) estimate that 22% of the global disease burden is due to preventable environmental factors, defined by the WHO as "the physical, chemical and biologic environment to the human host and related behaviour, but only those parts that could reasonably be modified" [51]. The human body's introduction to this novel chemical excess is increasingly associated with chronic inflammatory disease, such as inflammatory bowel disease (IBD), diabetes, obesity, cardiovascular disease, autoimmune disease, cancer, and neurodegenerative disorders [14].

Despite the safe status granted, many common food additives are increasingly recognized for their relevant effects in the etiology of chronic inflammation and disease [25]. Ultra-processed food consumption is linked with increasing chronic disease, and specific food additives have been found to promote inflammation [52]. For example, emulsifier consumption is linked to incidence of Crohn's disease on national and continental scales, which is hypothesized to be caused by increased bacterial translocation across the GI epithelium [53]. UFCs also have strong epidemiological links to inflammatory disease. Many common pesticides and POPs are detrimental to human health and are linked to a plethora of human chronic diseases, including IBD [54–58].

In this section we present evidence for the effects of UFCs and IFAs on the GI tract. Primarily, these effects appear to be disruption and modification of the GI microbiota, damage to the integrity of the GI barrier, and induction of cellular stress.

Environmental factors induce GI dysbiosis

A key impact of environmental factors on the GI microbiota is induction of dysbiosis. Dysbiosis of the GI microbiota is strongly linked with immune dysregulation and chronic inflammation and may contribute to development of IBD, autoimmune disease, cardiometabolic disease and cancer [59]. GI dysbiosis can enhance pathways to inflammation due to increased mucus breakdown, disrupted GI barrier integrity, altered metabolite production, increased oxidative stress, systemic dissemination of commensals, and increased pathogen infection [60–62]. There is a range of evidence highlighting the role of IFAs in modifying the GI microbiota, including sweeteners, emulsifiers and bulking agents. The ingestion of artificial sweeteners saccharin, sucralose, and aspartame appears to be associated with glucose intolerance due

to alterations to the GI microbiota [25]. Neotame, another artificial sweetener, can cause modification of GI microbiota, while Splenda can cause GI dysbiosis and GI inflammation [60], [61]. Concerning emulsifiers, a recent comprehensive study screened the impact of twenty commonly used emulsifiers on human microbiota, finding they could impact microbiota density, composition, expression of microbiota-derived pro-inflammatory molecules, and the metatranscriptome [65]. Therein, certain compounds could impact both microbiota composition and microbiota gene expression, with others capable of impacting microbiota gene expression alone.

Two of the most used emulsifiers in the food industry, carboxymethylcellulose (CMC) – a polysaccharide, and polysorbate-80 (P80) – a synthetic polymer, were both shown to alter microbiota composition of young and adult mice, including decreasing health-associated bacteroidales and increasing levels of mucolytic bacteria [61]. Modulation of the composition of microbiota by P80 was also detected in an ex vivo complex human GI microbiota model, while both CMC and P80 modulated microbiota gene expression [66]. Moreover, in vitro application of P80 to membranous cells (M-cells) induced a 59-fold increase in *E. coli* translocation, while soluble plant fibres caused a decrease [67].

It has been suggested that maltodextrin (a modified starch) can also support a similar phenotype, as consumption can enhance biofilm formation of adherent-invasive *E. coli* and their adhesion to intestinal epithelial cells [68]. Lastly, artificial sweeteners can also induce similar activity, as they enhance the ability of *E. coli* and *E. Faecalis* to form biofilms and adhere to, invade, and kill human intestinal epithelial cells [69]. These findings are highly relevant for human GI health, as *E. coli* adhesion and translocation across the epithelial barrier is associated with Crohn's disease [67]. In addition, a study in pigs (a physiologically superior

GI model to other non-primates) found carrageenan could induce dysbiosis, with comparable GI microbiota composition to human IBD patients [70]. Furthermore, carrageenan has been found to act as an adjuvant by inducing M1 macrophages during cancer immunotherapy [71]. The impact of carrageenan is relevant because it is highly consumed, for example, the average American citizen consumes 250mg of carrageenan every day in a range of food products where it functions as a thickener and bulking agent [52]. For further reading, Laudisi et al. have published a comprehensive review of the impacts of food additives on GI homeostasis [72].

Several studies have shown that both POPs and pesticides are able to induce multiple characteristics of IBD (including dysbiosis), through a range of pathophysiological activities. One looked at the effect of relatively low doses of six common dietary POPs on human faecal microbiota. Strikingly, all conditions induced pollutant-dependent gene expression while select conditions enriched the volatolome (volatile organic compounds) with sulphur compounds, and in TC7 cell culture – a human intestinal epithelial cell model, several POPs significantly increased IL-8, a potent pro-inflammatory mediator [13,73]. In addition, a separate study found that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) significantly increased expression of 13 antimicrobial resistance genes in mice GI microbiota, including 30 days after cessation of dosing [74]. Many studies show polychlorobiphenyls to induce GI inflammation and dysbiosis of the GI microbiota, in weanling offspring mice this resulted in GI dysbiosis and increased intestinal inflammation [75,76].

Other non-POPs, such as organophosphate pesticides, also disturb GI homeostasis through induction of dysbiosis, such as chlorpyrifos [77–79]. Moreover, administration of glyphosate

(at a dose considered safe) can modify the gut microbiota of rats in early development, and notably, administration of glyphosate-based herbicide Roundup produced a significantly different microbiota profile, suggesting synergistic effects of the adjuvant formula [80]. Many other pesticides also modify the GI microbiota, including aldicarb, leading to increased pathogenicity of the microbiota [81]; carbendazim, leading to inflammation, hepatic lipid metabolism disorder, and liver damage [82]; diazonon, leading to activation of stress response pathways [83]; and many more [84]. Thus, exposure to commonly used organophosphorus pesticides can promote conditions of GI dysbiosis. Microplastics themselves may also modify the GI microbiota, as they have been found to modify its function in marine medaka (*Oryzias melastigma*) and act as a carrier for pathogenic bacteria into loggerhead sea turtles (*Caretta caretta*) [85,86]. Lastly, AGEs have been extensively studied, with overwhelming evidence to support their modification of the GI microbiota across a range of in vitro and in vivo human/animal studies, as reviewed by Snelson and Coughlan [49].

Environmental factors increase gastrointestinal barrier permeability

Deterioration of the GI barrier – mechanical, chemical, biologic, and immune – allows unmetabolized peptides and other xenobiotic factors to infiltrate the lymph system and systemic circulation, which may elicit an immune antigenic response [87]. Importantly, increased GI barrier permeability is an important step in the onset of inflammatory disease [88]. For example, in IBD, adherens junction protein E-cadherin and tight junction proteins claudin-1, occludin, and zonula occludens (ZO)-protein-1, are downregulated by tumour necrosis factor (TNF) and interferon (IFN)γ, causing increased barrier permeability to

pathogens and other xenobiotics [88]. Therefore, identification of environmental factors that induce this phenotype is of relevance in the onset and maintenance of chronic GI inflammation.

In addition to the impact of GI dysbiosis, both IFAs and UFCs can also directly increase the permeability of the GI epithelium by decreasing tight junction protein expression or disturbing tight junction distribution [89]. Many food additives have been shown to induce hyperpermeability, such as glucose, salt, emulsifiers, organic solvents, gluten, microbial transglutaminase and nanoparticles [89]. Common artificial sweeteners saccharin, sucralose and aspartame were found to induce cell death and apoptosis in intestinal epithelial cells, while decreasing expression of claudin-3 – associated with better tight junction sealing and increasing claudin-15 – associated with pore formation and leakage [90]. Maltodextrin has been shown to disrupt the GI barrier in another way, by decreasing mucin-2 in the GI mucosal layer [72].

The surfactant properties of emulsifiers are generally predicted to decrease the hydrophobicity of the GI mucus layer, which can cause damage to the integrity of the epithelial barrier [89,91]. Furthermore, there is evidence that through impairing the GI barrier and altering the GI microbiota composition, emulsifiers could contribute to chronic low-grade inflammation and incidence of IBD and metabolic syndrome [92]. In Chassaing et al.'s study on the emulsifiers carboxymethylcellulose (CMC) and polysorbate-80 (P80), both induced hyperpermeability in the GI barrier [61]. The administration of CMC and P80 induced a reduction in mucus thickness and higher contact of bacteria with the epithelium. There was also an increase in serum antibodies to LPS and flagellin, signifying systemic dissemination due to the emulsifier-induced disturbances to GI homeostasis. In a follow-up study, sustained

exposure to CMC and P80 maintained a pro-inflammatory environment in the colon and contributed to carcinogenesis [93]. Furthermore, this effect was linked to thinning of the mucus layer, and increased serum expression of pro-inflammatory chemokines CXCL2 and CXCL1 [93]. This is particularly relevant to IBD, as CXCL1 is a potent neutrophil chemoattractant significantly elevated in IBD patient sera [94].

POPs also target intestinal epithelial cells, which are essential for maintenance of normal GI homeostasis [95]. For example, TCDD was found to modify jejunal epithelial cell gene expression, leading to alterations in uptake of luminal metabolites and metabolism [96]. Interestingly, TCDD is a known immunosuppressant and can increase susceptibility to a range of infections [74]. Consequently, TCDD elicits multiple activities that contribute to a chronic inflammatory phenotype. Other POPs induce similar disruption of the GI barrier. In adult and weanling offspring mice a range of polychlorobiphenyls induced GI inflammation, and developmental exposure to a panel of 12 polychlorobiphenyls commonly detected in serum of human mothers altered GI physiology and significantly increased tight junction permeability [75,76]. It is also suggested from a study on human Caco-2 cells that polychlorobiphenyls can downregulate ZO-1 and occludin expression [97]. Therefore, common dietary POPs disrupt the integrity of the GI barrier.

Non-POPs, such as organophosphate pesticides also induce GI barrier defects. For example, administration of chlorpyrifos to mice caused intestinal inflammation and abnormal intestinal permeability, associated with a “remarkable” increase in serum LPS [98]. Chlorpyrifos was shown in vitro and in vivo to impair GI epithelial cell tight junction protein ZO-1, and significantly decrease mRNA expression of claudin-1, occludin, and ZO-1 [77,99,100]. Consequentially, GI permeability induced by chlorpyrifos led to increased LPS levels in the

plasma, which induced upregulation of pro-inflammatory mediators in the liver (TLR-4, TNF-α) and adipose tissues (TNF-α, MCP-1, IL-1β, PAI-1) of mice [77].

Environmental factors increase GI oxidative stress and ER stress

The induction of unresolvable endoplasmic reticulum (ER) stress and oxidative stress is a major marker of chronic inflammatory diseases, including IBD, and is a stimulant for production of pro-inflammatory cytokines such as IL-1β and TNF-α, and cyclooxygenases [101]. UFCs and IFAs have been extensively shown to induce multiple mechanisms of cell stress, leading to increased inflammatory markers, although more research into these effects within the context of the GI microenvironment is needed. The impact of IFAs on oxidative stress and ER stress is exemplified by maltodextrin and aspartame. A maltodextrin diet can promote chronic low-grade inflammation via upregulation of IL-1β and induce p38 MAPK-mediated ER stress [72]. Other studies have also linked maltodextrin with intestinal stress and inflammation, labelling it a 'modern stressor of the intestinal environment' [102]. Furthermore, aspartame increases ROS production and oxidative stress in intestinal epithelial cells [90]. Titanium dioxide microparticles also induce ROS production in human intestinal epithelial cells in addition to activation of NLR family pyrin domain containing 3 (NLRP3) [103].

Considering UFCs, pro-inflammatory signals can be enhanced by dietary intake of advanced glycation end-products (AGEs), in addition to increased endogenous AGE formation that occurs in response to oxidative stress [104]. In turn, AGEs can act as catalysts for further ROS production, forming a positive feedback loop [49]. Past studies on dioxins and PFAS – both

detectable in human serum – found significant increases in ROS markers of oxidative stress, while PFAS also lowered total antioxidant capacity; highlighting the possibility that PFAS contribute to cytotoxicity [105]. Like other POPs, organochlorine pesticides such as β-hexachlorocyclohexane are also able to induce oxidative stress [106,107]. Furthermore, β-hexachlorocyclohexane was found to impair hepatic mitochondrial function in mice through downregulation of genes involved in fatty acid β-oxidation, in addition to reducing tricarboxylic acid (TCA) cycle metabolites malate and fumarate [38].

Exposure to organophosphorus pesticides such as chlorpyrifos and diazinon, and carbamate pesticide propoxur has been associated with acute and chronic health effects, such as the induction of oxidative stress, ER stress and GI dysbiosis [55,108]. Chlorpyrifos also significantly reduces expression of powerful antioxidants, superoxide dismutase and catalase [55]. In addition, Roundup was found to induce oxidative stress and cell death in prepubertal rat testis by a necrotic mechanism induced by ER stress and subsequent calcium influx into the cytosol [109]. Thus, commonly exposed POPs and pesticides can both elicit a cellular stress response, producing elevated levels of ROS. Together these results show that UFCs can induce oxidative stress and/or ER stress. Therefore, these findings provide further evidence for the role of ongoing IFA and UFC exposure environmental factors in the onset and maintenance of chronic inflammation.

An important consideration for the cellular stress induced by exposure to such environmental factors is the induction of major pro-inflammatory regulators. During ER stress, the ER produces 25% of all cellular ROS, which is sensed by NLRP3 and upon activation forms the NLRP3 inflammasome, a potent inflammatory activator [110]. The NLRP3 inflammasome is

proposed to act as a signal integrator, by detecting perturbations in cytoplasmic homeostasis, termed 'homeostasis-altering molecular processes' (HAMPS) [111]. Oxidative stress and ER stress induction cause drastic disturbances to cellular homeostasis, which could implicate NLRP3 inflammasome activation via HAMP detection. Indeed, it has been found that ROS is needed for NLRP3 inflammasome activation [112]. Therefore, it is proposed that environmental factors could promote NLRP3 activation in intestinal epithelial cells and immune cells by several pathways (Figure 3): increased adherence of bacteria to the GI epithelium, linked to modification of mucus production; GI barrier hyperpermeability, more pathogen/microbial-associated molecular patterns (P/MAMPS) will reach immune cells in the lamina propria; activation of CXCL1 and CXCL2, two chemokines that drive NLRP3 activation; induction of ER stress, production of excessive ROS, and release of calcium into the cytoplasm; induction of oxidative stress; and mitochondrial dysfunction [111,113]. Although ER stress also induces apoptosis via a JNK-MAPK-NF-κB pathway, it can activate the NLRP3 inflammasome that is associated with both apoptosis and pyroptosis, which itself leads to release of alarmins termed damage-associated molecular patterns (DAMPS) [114]. Altogether, since NLRP3 inflammasome can be activated by multiple signalling and cellular events, there is strong evidence to suggest that NLRP3 inflammasome activation could be implicated in the effects of the environment factors described in this review.

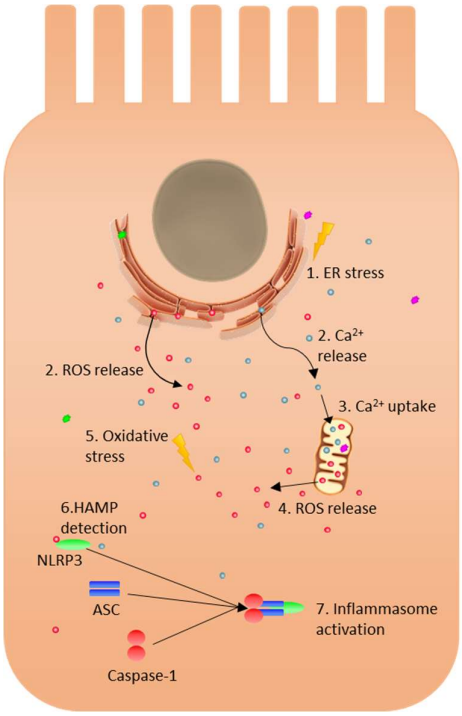

Figure 3 – A mechanism of environmental factor induced NLR family pyrin domain containing 3 (NLRP3) inflammasome activation. (1) Environmental factor induced endoplasmic reticulum (ER) stress leads to (2) release of calcium and reactive oxygen species (ROS) from the ER lumen into the cytoplasm. (3) Mitochondrial calcium uptake, combined with environmental factor induced mitochondrial dysfunction leads to (4) increased ROS production and release from the mitochondria into the cytoplasm (5) inducing oxidative stress. (6) Homeostasis-altering molecular processes (HAMP) detection (7) activates NLRP3 inflammasome formation with adaptor-apoptosis-associated-speck-like protein containing a caspase recruitment domain (ASC) and caspase-1.

Mechanism of induction and maintenance of chronic inflammation by environmental factors

The main objective of this review was to understand the impact of environmental factors on GI homeostasis and GI inflammation. We hereby propose a mechanism to illustrate environmental factor-induced and maintained GI inflammation (Figure 4). Considering the routine detection of UFCs in food, and the ever-increasing consumption of IFAs in (ultra-) processed food, persistent human exposure is a likely scenario. Thus, over time the full range of effects could become chronic, namely; GI dysbiosis, GI barrier defects, epithelial cell death, microbial translocation, GI and systemic pro-inflammatory signalling, plasma LPS, pro-

inflammatory immune cell recruitment and activation, oxidative stress, ER stress, and potential NLRP3 activation.

Ultimately, the immune system plays a vital role in our health, representing an innate sensor that serves to maintain the integrity and homeokinetic efficiency of our internal system [115]. Unresolvable disturbances to this system lead to dysregulation of the neuroendocrine-immune balance and a loss of immune homeostasis [1]. In the intestines, disturbance to homeostasis – through dysbiosis and hyperpermeability – is proposed to be an initiating factor in sterile low-grade systemic chronic inflammation [116]. This review provides strong evidence that persistent exposure to, and bioaccumulation of, IFAs and UFCs can sustain mounting, unresolvable and lifelong disturbances to the complex network of the GI microenvironment and beyond. The capacity of these environmental factors to induce a state of GI dyshomeostasis may be further illustrated by considering the different dose-dependent effects, for example different dose exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin triggered regulatory or pro-inflammatory CD4+ T cell differentiation [117].

A further important consideration for the activities described is the plausibility of an additive effect, whereby persistent exposure to (interchanging) factors may maintain chronic GI inflammation. The size of the exposome and variety of effects associated with IFAs and UFCs indicates a broad range of stimulus-specific signalling activity and gene expression, which is believed to play an important role in the robustness of the immune system, but also in pathogenicity [118]. Moreover, there are further impacts of the effects described in this review, for example GI dysbiosis is suggested to fuel IBD-associated genetic changes [119].

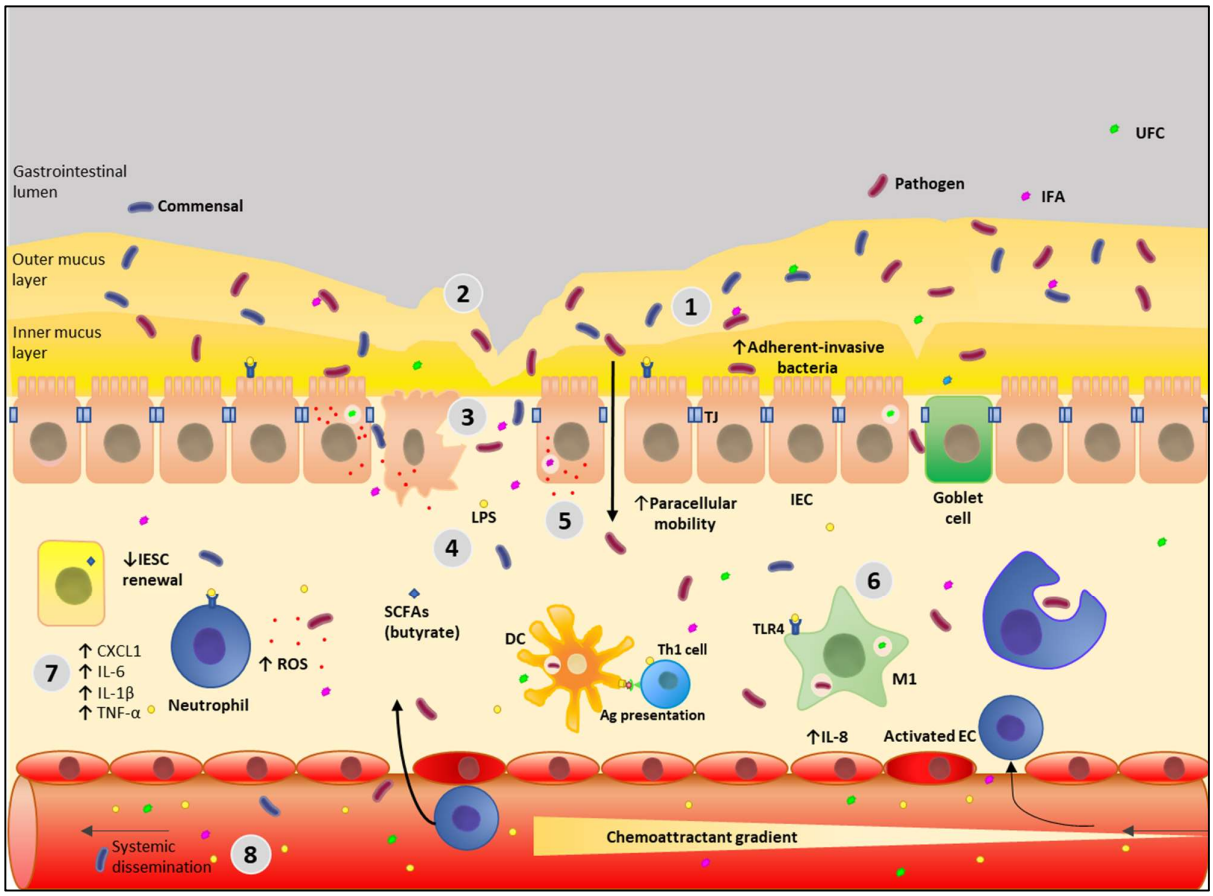

Figure 4 – Mechanism for onset and maintenance of environmental factor induced chronic inflammation in the gastrointestinal tract. Multiple steps may occur concurrently, such as: **1** – Induction of gastrointestinal dysbiosis, associated with increased mucolytic bacteria and adherent-invasive bacteria. **2** – Depletion of mucus by mucolytic bacteria and decreased mucin-2 expression. **3** – Hyperpermeability due to downregulation of tight junction proteins and IEC cell death, with decreased renewal by IESCs. **4** – Increased infiltration of commensal and pathogenic bacteria, endotoxins and environmental factors. **5** – Cellular stress (endothelial stress and oxidative stress). **6** – Immune cell activation and recruitment, endothelial cell activation. **7** – Expression of pro-inflammatory cytokines. **8** – Increased systemic dissemination of pathogens, endotoxins and environmental factors. Unintentional food contaminant (UFC), intentional food additive (IFA), dendritic cell (DC), endothelial cell (EC), T helper-1 cell (Th1), classically activated macrophage (M1), intestinal epithelial cell (IEC), intestinal epithelial stem cell (IESC), toll-like receptor (TLR-)4, lipopolysaccharide (LPS), tight junction (TJ). Reactive oxygen species (ROS – red dots).

Environmental factors and sub-system resilience

Deterioration of the GI sub-system is accompanied by uncontrolled infiltration of infectious agents and other environmental factors (such as IFAs, UFCs), as well as dietary components (such as free fatty acids) and digestive enzymes [120]. This review found evidence that environmental factors can cause deterioration of GI health. Across a range of animal studies in multiple species, UFCs and IFAs increased the antigenic load locally *and* systemically, causing pro-inflammatory responses at distal sites such as the liver, and metabolic syndrome and persistent low-grade inflammation in adipose tissue [61,77,93]. Furthermore, dissemination of environmental factors in the plasma presents further routes for disruptions, for example, pesticides are known to target tight junction proteins of the blood brain barrier, increasing its permeability [121]. This illustrates the subsequent impact of environmental factor-induced deterioration of the GI tract on the resilience of multiple other physiological sub-systems [122].

In humans many well studied scenarios illustrate the intimacy of distinct physiological sub-systems, for example, following heart surgery GI barrier function is impaired leading to increased LPS in plasma [120]. The findings we have presented in this review suggest environmental factors contribute to the induction of a 'tipping point' in the GI tract; a point at which further deterioration becomes self-propagating, generating a contrasting pathological GI state [123]. Consequentially, beyond IBD, the findings of this review are relevant to the study of multimorbidities as 'complex adaptive systems responses', as the evidence herein suggests exposure to external environmental factors can perturb

interconnected biological processes and immune homeostasis, and dysregulate stress systems, control of gene expression and mitochondrial function [115].

Regulatory questions

Considering the prevalence of exposure to environmental factors, and the low concentrations at which this can impact upon physiology, it is important to scrutinise existing regulations regarding their use. For example, IFAs are inconsistently regulated across the FDA and EFSA – the two major food safety regulators worldwide. In the USA, the early approval of over 300 food additives has not been re-evaluated since initial safety tests were carried out in the 70's – 80's [72]. However, in the EU extensive re-evaluations are nearing completion. For example, carrageenan was recently given an updated acceptable daily intake recommendation [124]. However, due to inadequate data – including on the possible role of carrageenan in IBD pathophysiology – this must be re-evaluated within five years. Therefore, this highlights the need for further research into the possible role of IFAs in chronic inflammatory disease. Given the significant number of IFAs consumed, especially in ultra-processed food, future studies must also focus on combinatorial effects, along with the health impact of cumulative lifelong intake. Such research is essential, as consumption of ultra-processed food is associated with increased risk of IBD worldwide [125].

Regulation of UFCs such as pesticides must also face scrutiny, as certain aspects of their use and activity are not considered in toxicology tests. Firstly, safe limits of active ingredients in pesticides are always determined in isolation, and do not account for possible synergistic effects with other chemical substances [126]. Secondly, only the active ingredient in plant

protection products is required to be toxicologically tested, as the rest of the mix is considered proprietary business information – even though all ingredients, such as adjuvants, are relevant for rigorous toxicological study [127]. Despite the inertia surrounding regulation of adjuvants in pesticides, they are well documented to have toxic effects on human health, and their omission from toxicology tests is suggested to "falsify the safety profile of commercial pesticides" [128]. In the case of Roundup, the full mix is 125-fold more toxic than glyphosate – the active ingredient – alone, while the adjuvants were found to significantly enhance endocrine and toxic effects in mammals and human cell lines [129,130]. Therefore, all compounds included in pesticide mixes should be subject to toxicology testing, not only the advertised active ingredient.

Lastly, open questions remain surrounding the toxicology of degradation products from pesticide chemicals and POPs, as well as IFAs such as artificial sweeteners, which arise from metabolization by the human GI microbiota [131,132]. Metabolization of POPs has been found to produce active oestrogenic molecules, which can target many different immune cells, leading to effects such as increased neutrophil numbers in the spleen of mice [133,134].

Conclusion

The findings presented herein join a growing body of work that seeks to bring attention to the probable role of ingestible environmental factors in the onset and maintenance of a chronic inflammatory state in the GI tract. Firstly, there is a significant body of evidence describing the impact of environmental factors on the GI microbiota, both in terms of density and composition, such as through increasing levels of mucolytic bacteria, or adherent-

invasive *E. coli*. Secondly, the same or different environmental factors produce a range of GI barrier disrupting effects, from reduced mucous production to downregulation of tight junction proteins. Thirdly, through induction of cellular stress and increased inflammatory signals in response to infiltration across the GI mucosa, the activation of the major inflammatory regulator, NLRP3 inflammasome, is proposed. The common effects induced by the multitude of IFAs and UFCs likely lead to additive effects, whereby substitution of interchangeable factors fuels continued disruption to GI and immune homeostasis. Moreover, there is evidence that GI dyshomeostasis induced by environmental factors can cause consequent deterioration of additional distinct physiological sub-systems in the body.

Greater research is needed on the physiological effects of environmental factors in the GI tract to better regulate their approval and usage. Further research will also provide more clarity around the role of environmental factors such as IFAs and UFCs in chronic inflammatory diseases, such as IBD.

Acknowledgements

We would like to thank professor Marjolein van Egmond for her support.

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