

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Statement on interactions between xenobiotics and the human microbiota and their potential toxicological implications.

Introduction

1 The term human microbiota refers to the population of microorganisms (bacteria, viruses, fungi, archaea, protozoa) living in internal compartments and on the surface of human beings. This statement aims to describe the current state of knowledge of changes in the population and function of the gut microbiota¹ caused by exposure to components of, and contaminants present in, the diet and the effects of the gut microbiota in modulating the toxicity of those substances.

2 There is a substantial body of literature on the microbiota, therefore this paper has used relatively narrow search terms and thus is a representative rather than comprehensive survey of the microbiota and their interaction with ingested xenobiotics

Composition and distribution of the microbiota

3 The externally accessible compartments of the human body are inhabited by microorganisms. By far the greatest number and variety of microorganisms are present in the digestive tract, predominantly in the caecum. The most studied of these are bacteria that fall into the phyla of the Gram-negative *Bacteroidetes* and the Gram-positive *Firmicutes*. Other relatively abundant phyla are the *Actinobacteria*, the *Proteobacteria* and the *Verrucomicrobia* but the full range of species varies from site to site, from individual to individual and with diet (David *et al*, 2014) health, sex, ethnicity, age and geographical location. This statement concentrates on the bacterial population of the microbiome since the majority of the scientific literature relates to them. The terms microbiome (defined more precisely below) and flora are also used as synonyms for the microbiota in the literature as well as in this Statement.

4 Although estimates vary, the number of organisms in the gut appears to exceed that of human cells in the whole body. Estimates of microbe/human cell ratios have decreased in recent years from 10:1 to about 1.3:1 but this depends upon the definition of a cell, for example, whether erythrocytes and platelets can be considered as true cells (Sender *et al*, 2016). The commonly cited figures for the number of species represented are 5,000 to 10,000, and sometimes many more, , although an individual may harbour many fewer species than this. The gene set of

¹ Unless stated otherwise, the general information on the gut microbiota in this introduction (paragraphs 1 – 25) is taken from reviews by Rowland *et al* (2018), Jandhyala *et al* (2015) and Hollister *et al* (2014).

the gut microbiota – the precise definition of the term (gut) microbiome – is estimated at about 3 million genes, or about 150 times that of the human genome. All of the above values are central estimates and numbers will vary between individuals.

5 Identification of the composition of the microbiota was originally problematic because many of the bacteria are obligate anaerobes and/ or have precise pH, temperature or nutrient requirements and could not easily be cultured. Since the mid-1980s, techniques for identifying bacterial communities from phylum to species level have improved dramatically in speed and efficiency. Sequencing the ribosomal 16S RNA gene has picked out stable and variable regions that can be used as a “fingerprint” to identify unculturable bacteria. The second genomics technique in use is Whole Metagenome Shotgun (WMS) sequencing. These methods cannot discern active from dead or quiescent organisms. High throughput “next generation” sequencing developed in the early 2000s has reduced sequencing time from months or years to hours or days. Large multinational research projects such as the European Metagenomics of the Human Intestinal Tract (MetaHIT) and the American Human Microbiome Project (HMP) have produced extensive data on the microbiome in relation to health and disease. Transcriptomics, proteomics and metabolomics have all been used to identify the gene expression and functions performed by the microbiota. (Review by Hiergeist *et al* (2015)).

6 Raymond *et al* (2019) compared different culturing techniques with culture independent methods for characterising the human gut microbiota with regard to bacteria carrying antimicrobial resistance (AMR) genes. They were able to discern the difference between bacterial species carrying AMR genes in their main band DNA from those that carried them in transposable elements and, those AMR genes that were related to essential functions of the bacteria and those that were not.

7 The composition of the gut microbiota of new-borns appears to be influenced to some extent by the method of birth. Babies born by vaginal delivery acquire gut bacterial populations similar to those in their mother’s gut and vagina while babies born via Caesarean section (C-section) acquire predominantly skin surface bacteria, (Milani *et al*, 2017). Yatsunenکو *et al* (2012) found that the phylogenetic composition of the bacterial community evolved towards an adult-like configuration within the 3-year period following birth, although a later study by Holister *et al* (2015) found that pre-adolescent children have a more diverse gut population than adults, and suggested that development to the adult state was a more gradual process).

8 The first stool of C-section neonates has been found to have fewer microbial genes associated with amino and nucleotide sugar metabolism and more related to fatty acid metabolism, amino acid degradation and xenobiotic metabolism, with reduced bacterial diversity compared with those born vaginally (Mueller *et al*, 2017). However, as reviewed by Rodriguez *et al* (2015), some of the microbiota in the new-born gut appear to be present at birth, and the meconium harbours a population dominated by *Staphylococci* and *Lactobacilli*, as distinct from the first true faeces, which is richer in *E. coli*, *Enterococcus* and *Klebsiella pneumoniae*. It has been postulated that bacteria from the maternal gut access the amniotic fluid via the blood and thence colonise the fetal digestive tract.

9 Changes to the relative composition of the gut microbiota, possibly leading to the overgrowth of normally relatively minor taxa and thence to a possible change in the balance of functions, is known as dysbiosis. However, the term implies some detrimental alteration, which may have downstream effects on the health of the digestive tract and the individual as a whole, whereas variation in the balance of the microbial population may be adaptive or age related and be neither harmful nor indicative of harm done to the host (Undark, 2019). The term dysbiosis is used in this paper to indicate a change in the make-up of the microbiota and where an author remarks on correlation with, for example, a disease state, then this may be noted but no causation is implied.

10 Changes in the ratio of *Firmicutes* to *Bacteroidetes* ratio are frequently used in the literature to indicate dysbiosis, possibly caused by an ingested substance or disease state. Ley *et al* (2005) found that in homozygous genetically obese (ob/ob) mice, the population of the *Firmicutes* (*F*) was significantly increased and that of the *Bacteroidetes* (*B*) significantly reduced ($p < 0.05$) relative to both wild-type (wt/wt) and heterozygous (ob/wt) mice. The authors could not discern whether this difference was a cause or an adaptive consequence of obesity. Koliada *et al* (2017) also found that adult humans had a *F/B* ratio that increased in a manner that was significantly associated with individuals' body mass index ($p < 0.005$). However, these authors also pointed out an earlier study by Schwartz *et al* (2010), who found that a reduction in the *F/B* ratio was associated with being overweight in otherwise healthy human volunteers. In addition, Mariat *et al* (2009) reported that the *F/B* ratio in humans changed significantly with age, such that its median value in infants (aged 3 weeks to 10 months) was found to be 0.4, in adults (25 to 45 years) 10.9 and in elderly people (70 to 90 years) 0.6, indicating natural age-related changes in the make-up of the microbiota, but with apparent stability in the middle years.

11 Scepanovic *et al* (2019) investigated the effects of host genetics and demographic/ environmental factors on the diversity of the gut bacterial flora of 1000 healthy volunteers (500 men, 500 women, 200 subjects in every decade from 20 to 70 years of age). Volunteers' stool samples were subjected to 16S rRNA gene analysis, detailed demographics questionnaires were completed, and blood samples were genotyped. Bacterial diversity showed much higher correlation with lifestyle factors than with genetic factors. Diversity was known to increase with the consumption of fruit and fish and decrease with fried food, but this study also went deeper and, for example, associated the species *Clostridium papyrosolvens* of the *Firmicutes* with the oral intake of mineral supplements and the presence of the family *Comamonadaceae* with the age of subjects. To fully address the apparent lack of a significant association between host genetic factors such as SNPs and microbiome composition, however, the authors recommended using shotgun metagenomics instead of 16S rRNA profiling and pooling large data sets.

Non-bacterial components of the gut microbiota

Fungi

12 The fungi that inhabit the digestive tract constitute about 0.1% of the total microbiota and are known collectively as the mycobiota. A range of species has

been identified, largely within the *Ascomycota* and *Basidiomycota* phyla, dominated by *Saccharomyces*, *Malassezia* and *Candida* species. Like the gut bacteria, many fungal species have been found to be difficult to culture, so techniques for identifying them have included 18S rRNA gene sequencing (the eukaryotic equivalent to the 16S rRNA sequencing used for the bacteria) and Internal Transcribed Spacer (ITS) sequencing, which have provided analogous results. Nash *et al* (2017) compared the faecal mycobiome of 147 healthy HMP volunteers and found great variability between individuals but no correlation of composition with a long list of variables including age, sex, BMI, ethnicity and tobacco use. Among the 247 genera identified, the universality of the major species *S. cerevisiae*, *M. restricta* and *C. albicans* suggested that they may be resident commensals, but the possibility could not be ruled out that these fungi were present due to regular inoculation from the environment or the diet. Hallen-Adams and Suhr (2017) also reviewed work on the healthy mycobiome and highlighted measured differences due to diet and the difficulties involved in ascribing the origin – commensal or contaminant – to fungi in faeces samples.

13 Conversely, Strati *et al* (2016) found significantly higher numbers of fungal isolates and species in females compared with males ($p < 0.005$) in a cohort of healthy Italian volunteers (49 male and 62 female). Moreover, while diversity was not linked to sex, there was a decline in diversity from infants and children to adults. Most isolates grew *in vitro* at 37°C but were progressively inhibited at temperatures above 40°C and were sensitive to low pH (2) but grew as well at pH 3 as at pH 6.5. The presence of bile from 0.5 to 2.0% in the culture did not inhibit growth. The results of these stress tests indicated that the isolates could survive colonisation conditions in the human gut. Some isolates were resistant to fungicides, but others were not.

14 Schei *et al* (2017) investigated the possibility that, as with bacteria, the gut mycobiota of new-borns was transferred to them from their mothers. The occurrence of fungi in offspring appeared to mirror, to some extent, that of their mothers but there was no observed difference between children born vaginally and by C-section. The authors reported what they claim to be the first finding of *S. cerevisiae* in the infant gut and note that this becomes more established after initial consumption of yeast-rich food such as bread. Other fungi originated from breast milk and yet others such as those of the *Agaricomycetes*, were recognised as being of environmental origin.

15 Various fungi have been found to interact with members of the bacterial microbiota and have been associated with maintenance of homeostasis of the gut as well as with conditions such as obesity, carcinogenesis, irritable bowel syndrome, inflammatory bowel disease and Crohn's disease (see papers and reviews by Sam *et al* (2017), Luan *et al* (2015), Paterson *et al* (2017), Gu *et al* (2019) and Rodriguez *et al* (2015)). Associations have been reported between the composition of the human intestinal mycobiota and visceral hypersensitivity in IBS patients (Botschuijver *et al*, 2017, from abstract), uveitis (Jayasudha *et al*, 2019) and neurological diseases such as multiple sclerosis and motor neurone disease (Forbes *et al*, 2019).

16 Forbes *et al* (2019) published a pair of graphs illustrating the growth of publications per year relating to microbiome and mycobiome studies. Between 2005 and 2009 the number of microbiome papers was scarcely out of single figures per year, but then publication rate increased sharply such that there were over 3500 papers published in 2018. For the mycobiome, conversely, the first paper was published in 2008. This was followed by an accelerating increase up to the last plotted measurement, again in 2018, of about 27 papers per year

Viruses

17 The viral content of the gut microbiota (the “virome”) is largely under-reported but may be equal in size or, in some niches, larger than the bacterial population. A variety of single- and double-stranded DNA and RNA viruses, including bacteriophages and known pathogens, have been found to inhabit the human gut, with acquisition apparently taking place postnatally through dietary, maternal and environmental contact.

18 Manrique *et al* (2016) discovered a healthy human phageome consisting of bacteriophages that were shared to varying degrees between individuals. This comprised a core population that appeared to be present in all individuals, a group that were common but not ubiquitous in a population and a third group that were less common. The authors speculated that most, if not all, of the bacteria in the human gut possessed one or more lysogenic phages and that the active phageome consists of these viruses in the lytic phase. In a previous study using a murine gut microbial ecosystem model, evidence was obtained for an important role for the phage population in modulating bacterial dynamics and Manrique *et al* (2016) presumed that the relationship would be similar in the human gut.

19 The presence of bacteriophages in the human gut microbiota appears to have advantages and disadvantages to the health of the flora and in the downstream health of the host. A number of reviews were found that cover the influence of phages on the acquisition of AMR genes by the bacteria, control of the balance of taxa, host immunity and involvement in the aetiology of metabolic and inflammatory diseases. (Aggarwala *et al* (2017), Santiago Rodriguez TM *et al*, (2019), Garmeva *et al* (2019), Zuo *et al* (2019), Lawrence *et al* (2019), Mukhopadhyaya *et al* (2019), Neil *et al* (2018), Carding *et al* (2017), Lim *et al* (2015), Ogilvie & Jones (2015), Minot *et al* (2011))

Archaea

20 The human gut (along with other niches) also plays host to members of the *archaea*, which are similar in morphology to bacteria but also possess eukaryotic traits as well as unique features. These organisms are relatively poorly characterised since most analyses of the microbiota are tailored towards identification of bacteria. However, a number of species have been associated with the human gastrointestinal tract, many of which are methane producers by various metabolic pathways, the most prevalent organism being *Methanobrevibacter smithii*. Positive and negative correlations have been found between the titres of the *archaea* and the gut bacteria, but no unequivocal correlations have been confirmed between the *archaea* and metabolic or inflammatory disease. See, for example, Gaci *et al* (2014), Hoffmann *et al* (2013), Koskinen *et al* (2017). Ramezani *et al* (2018) found that the presence of

M. smithii, which is known to metabolise trimethylamine to produce methane, in the gut of apolipoprotein e^{-/-} mice, reduced the blood plasma level of trimethylamine N-oxide, which is linked to the development of atherosclerosis. The authors recognised, however that this was a small study that would need to be followed up.

Protozoa

21 Berrilli *et al* (2012) reviewed the interactions between the bacteria of the human gut and eukaryotic microorganisms, treating separately protozoa, such as *Cryptosporidium*, *Entamoeba* and *Giardia* and helminths such as nematodes, flatworms and roundworms. Infestation with the protozoa may be symptomless but can also cause a spectrum of diarrhoeal disease from mild and self-limiting to severe and prolonged. Bacterial-protozoal interactions have been observed in mice to attenuate protozoal pathogenicity but may also exacerbate it. The presence of Gram-negative bacteria, such as *E. coli* has been shown to enhance the virulence of *Entamoeba histolytica*.

22 Many gut protozoa, for example *T. gondii* and *Giardia*, damage the epithelium and disrupt the mucus layer, which can allow microbes greater contact with and even translocation across the epithelial barrier. Conversely, helminths often promote epithelial regeneration and mucus production, through upregulation of host IL-22 production, helping to contain bacteria within the gut and limit their access to the epithelium. However, some helminth species, including *Strongyloides venezuelensis* and *Trichinella spiralis*, also appear to alter junctional proteins, sometimes at sites distant from those of parasite attachment, which can allow bacteria and bacterial lipopolysaccharide (LPS) into the portal circulation. Helminths and protozoa may thus have opposing effects on barrier function, but this may be species dependent (Review by Leung *et al*, 2018).

23 Lokmer *et al* (2019) used shotgun metagenomics to identify gut protozoa from individuals living in communities at various levels of industrialisation. The authors mapped 127 protozoan genomes from various countries (57 from Cameroon, 22 from Tanzania, 18 from Peru, 19 from the USA and 11 from Italy). The species *Blastocystis* was found in all populations, with the highest incidence in farmers from Cameroon. *Entamoeba spp* were not present in the subjects from Italy and the USA, but increased in non-industrialised populations, reaching up to 90% in Peruvian hunter-gatherers.

24 Parasites such as *Entamoeba histolytica*, *Giardia intestinalis* and *Trichomonas suis* are known to produce mucolytic enzymes that allow them access to the gut epithelium as part of their pathogenicity mechanism that have downstream effects on the interplay between the host and mucous-dwelling bacteria. In response to the presence of the nematode *Trichuris muris*, the mouse gut changes the expression of proteins in the mucosal layer, switching from MUC2 to MUC5AC and promotes the worm's egestion.

25 Literature searches pertaining to the effects of xenobiotics on the fungal, viral, protozoal and archaeal composition and function of the microbiota produced very few relevant papers, reflecting the current paucity of knowledge available on external influences on the lesser-investigated components of the gut flora. The remainder of

this paper therefore concentrates on the better characterised bacterial component of the gut microbiota.

Metabolites produced by gut microbiota

Short Chain Fatty Acids (SCFAs)

26 The metabolism of carbohydrates that are non-digestible by the host, such as inulin, by various bacterial genera in the gut leads to the production of short-chain fatty acids, primarily acetate, propionate and butyrate, with other lesser components, including branched-chain acids. Butyrate appears to be an important nutrient for the gut epithelium, as well as maintaining its barrier function and thus preventing “leakage” of gut antigens and pro-inflammatory molecules into the general circulation. Butyrate has been found to be effective in reducing the symptoms of ulcerative colitis in humans (Scheppach *et al* (1992)). However, Imai *et al* (2012, from abstract) found that cell-free supernatant from cultures of butyrate-producing bacteria found in the human gut, such as *Fusobacterium nucleatum*, *Clostridium cochlearium* and *Eubacterium multiforme*, could promote histone deacetylation *in vitro* and promote reactivation of latent HIV-1 infection.

27 Kimura *et al* (2013) investigated the action of SCFAs at adipose tissue-expressed GPR43 G-protein-coupled receptors in wild type and *Gpr43*^{-/-} mice. The knockout mice were obese, and the wild type were lean. Activation of the receptor was found to decrease insulin sensitivity and fat accumulation in adipocytes from white, but not brown, adipose tissue, and increase insulin sensitivity in muscle and liver. Acetate was found to suppress insulin-induced glucose and fatty acid uptake in adipocytes from wild-type but not *Gpr43*^{-/-} mice. Acetate moreover promoted phosphorylation of PTEN, a known downstream effector of GPR43, which blocks the insulin receptor cascade by dephosphorylating PIP₃. Thus, acetate suppressed the effect of insulin in adipose cells without directly affecting insulin receptors. The increase in insulin sensitivity in other tissues was thought to result from GPR43 activity increasing glucose uptake.

28 Oleskin and Shenderov (2016) briefly reviewed observed effects of SCFAs on host neurotransmitter function. Propionate and butyrate appeared to regulate expression of the gene for tryptophan hydroxylase, the rate-limiting step in serotonin synthesis, and decrease the activity of histone deacetylases, which appears to improve various neurological conditions, such as Parkinson’s disease, depression and schizophrenia. Hoyles *et al* (2018) reported that physiological concentrations of propionate had a protective effect on the permeability of the blood brain barrier against bacterial lipopolysaccharide as seen in an *in vitro* model system. The mechanism appeared to be downregulation of expression of the protein CD14, an accessory protein involved in the LPS activation of proinflammatory Toll-like receptors. Such beneficial actions of SCFAs appear to be concentration-limited since high concentrations, especially of propionate, had been associated with the expression of autism-related genes.

29 Acetate has also been found to mediate intestinal IgA release via activation of GPR43 receptors. This effect was not mimicked by butyrate and was independent of T cells. The promotion of IgA production by acetate was not through direct

stimulation of B cells but rather by activation of retinoic acid production of regulatory dendritic cells, which then induced B cells to produce IgA.

30 Morrison and Preston (2016) reviewed recent evidence for the influence of acetate, propionate and butyrate on gut integrity, glucose homeostasis, lipid metabolism, appetite regulation and immune function. They concluded that “The multifaceted roles of SCFAs suggest that they may play an important role over the life-course in protecting the body against deteriorating metabolic control and inflammatory status associated with Western lifestyles”.

31 SCFAs appear to be present in the colon at diet-dependant concentrations in the millimolar range (den Besten *et al*, 2013) and have been found to elicit different effects on gut immunity and other functions at different concentrations, as noted above for propionate, and as has been found for butyrate (Kespohl *et al*, 2017). SCFAs thus appear to be multifunctional effectors linking the metabolism of the gut microbiota to host physiology.

Bile acids

32 The steroidal metabolites of cholesterol conjugated with glycine (in humans) or taurine (in rats) that are produced by the liver are primary bile acids. Primary bile acids are released into the lumen of the duodenum via the bile duct and act as lipid emulsifiers, producing micelles around fats and promoting their uptake. Conjugated bile acids are not reabsorbed by the small intestine but are excreted in the faeces. The gut microbiota are capable of deconjugation, regenerating free steroids that can undergo enterohepatic circulation. The metabolites of the primary bile acids produced by the microbiota are termed secondary bile acids. Bile acids have hormonal actions throughout the body, particularly through the farnesoid X and GPBAR1 (also known as TGR5) receptors.

Others

33 The gut microbiota can synthesise B and K group vitamins, including biotin, cobalamin, folates, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine. Antibiotic treatment affects plasma prothrombin levels in people on a low-vitamin K diet. The *Bacteroidetes*, *Fusobacteria* and *Proteobacteria* appear to mostly account for these pathways, with lesser involvement of the *Firmicutes* and the *Actinobacteria*.

34 Roager and Licht (2018) reviewed the effects of bacterial metabolites of the amino acid tryptophan, arising initially from protein degradation, on host health. Tryptophan undergoes metabolism by the microbiota by oxidation, decarboxylation, diacylation, and amino transfer. The direct metabolites are processed further into products that act as signalling molecules (indole and 5HT), aromatic hydrocarbon receptor (AhR) ligands and effectors in inflammatory bowel disease.

35 Anaerobic choline metabolism by the microbiota produces trimethylamine (TMA), acetate and ethanol. Dysbiosis leading to aberrant choline metabolism has been proposed as a potential contributing factor in non-alcoholic fatty liver disease, and increased TMA in the circulation has been mooted as a risk factor for

cardiovascular disease and colon cancer. The choline utilization (*cut*) gene cluster in sulfate reducing bacteria is thought to be responsible for this pathway. This gene cluster in human gut bacteria encoding TMA-lyase (*cutC*) is widely distributed across different phyla, and the pathway may also have been acquired in some strains via horizontal gene transfer (Krishnan *et al*, 2015).

Prebiotics and probiotics

36 Prebiotics are foods or components in foods that are intended to act as substrates for “beneficial” bacteria, those that maintain the healthy functioning of the gut epithelium and restrict the growth of pathogenic species. Examples are inulin and oligofructose. Effects on the host resulting from the consumption of these substances appear to be reduction in blood very-low-density lipoprotein (VLDL), triglycerides and total cholesterol, reduction in gut inflammation and possibly protection against colorectal cancer (Markowiak and Slizewska 2017).

37 Some processed foods such as breakfast cereals may not be naturally high in insoluble fibre and so may be supplemented by the manufacturer with fibrous material from other sources. Gamage *et al* (2018) investigated three commercially available fibre products in the Australian market, namely, sugarcane stem, wheat dextrin and Psyllium husk. Sugarcane stem contained the highest amount of lignin and xylose, while wheat dextrin had the highest amounts of mannose and glucose, and Psyllium husk contained the highest amount of arabinose. Each product was subjected to a series of pH-controlled enzyme treatments to simulate human digestion, and the effect of each on the human gut microbiota was examined in an *in vitro* system with an anaerobic medium, to simulate the conditions in the human large intestine. Faecal samples were obtained from six healthy adults. Fibre-specific changes in the microbial community were observed, which were potentially linked to the chemical composition of each tested product. The abundance of *Bacteroidaceae* (genus *Bacteroides*) and *Porphyromonadaceae* (genus *Parabacteroides*) was significantly higher upon addition of wheat dextrin and Psyllium husk, while *Lachnospiraceae* and *Ruminococcaceae* were highly abundant in the presence of sugarcane stem. SCFA production increased with all of the fibres, but more so with the wheat dextrin and Psyllium husk than with the sugarcane, probably as a result of the bacterial population changes. The authors recommended that *in vivo* experiments could be performed to gain further insight into the long-term effect of fibre products on the gut microbiota and how long the changes might last after consumption, while taking into account differences in health, normal diet and colonic transit time between individuals.

38 Probiotics contain bacteria that are ingested with the intent of maintaining the balance of the microbial communities in the gut, maintaining the integrity of the epithelium and preventing the overgrowth of pathogens. *Lactobacillus* spp or *Bifidobacterium* spp are often added to yogurts and drinks with this stated intent. Fijan (2014) reviewed the field of probiotics and discussed health claims related to their use and concluded that care should be taken in their use by people with existing conditions such as leaky gut, compromised immune systems or critical illnesses, not least because their effects seem to be strain specific. It is, however,

possible for a suitable organism with resistance to stomach acid and bile to reach its intended target of the large intestine and exert some re-balancing effect on a microbial community in dysbiosis, even if the presence of that organism may itself be transient.

39 Additives to the diet that provide both a prebiotic nutrient and a probiotic bacterium are termed synbiotics. Markowiak and Slizewska (2017) list clinical trials with a range of bacteria and fructo-oligosaccharides. The studies showed improvements in a number of outcomes (biomarkers) associated with obesity, insulin resistance and type 2 diabetes.

40 However, studies on the effects of changes in the balance of the gut microbiota on health have largely been performed on animals and the responses of humans and experimental animals to the same substances may differ. Nguyen *et al* (2015) considered how informative the use of mouse models is in relation to research on the human gut microbiota. The authors considered the effects of anatomical, environmental and dietary factors on the composition of the mouse gut microbiota and the apparent differences from the human gut flora. Humanised murine models, i.e. originally gnotobiotic (germ free) animals whose colons were inoculated with human faecal samples, were recognised as having the research advantages of mouse models in general (life cycle, handling, genetic information, wide usage etc) as well as being a much closer approximation to the human situation than wild-type animals, but were known to develop changes in the balance of bacterial taxa simply because the recipients are not human. Although such models had been used for assessing functional changes in the microbiota that could not be easily or ethically be produced in humans, the lack of some taxa or changes in the taxonomic balance led to uncertainties as to how closely disease-related changes mimicked those in humans. Moreover, colonies of gnotobiotic mice are expensive and difficult to maintain.

41 Faecal transplants from obese to lean or from lean to obese mice have been shown to lead to the recipient developing the opposite phenotype, but this has not been proven to occur in humans. The prevalence of various taxonomic groups of bacteria in the human GI tract has been correlated with type-2 diabetes, obesity and other conditions but evidence of causality is stronger in some cases than others. Wortelboer *et al* (2019) reviewed current progress in the use of faecal transplants in the treatment of various conditions and disease states. Whereas recurrent or refractory *Clostridium difficile* infection is now recognised as being amenable to this treatment, the evidence for efficacy in inflammatory bowel disease, ulcerative colitis, irritable bowel syndrome, Crohn's disease and other conditions is hampered by variable results and the need for further studies. If the baseline involvement of the taxa of the microbiota in human pathology has not been established, then correlating disease states with the effect of xenobiotics and their metabolites on the gut taxa is even more problematic.

Functional aspects of the human gut flora

42 Tanca *et al* (2017) performed metagenomics and metaproteomics on stool samples from 15 healthy subjects in Sardinia to investigate potential and expressed functions of their gut flora. The *Bacteroidetes* were found to be involved in iron homeostasis, catabolism of non-glucose monosaccharides (rhamnose, xylose) and folate metabolism. *Firmicutes* were involved in butyrate synthesis, to a large extent by *Faecobacterium*, expressing the associated enzymes acetyl-CoA-C-acetyltransferase, 3-hydroxybutyryl-CoA dehydrogenase, butyryl-CoA dehydrogenase, glutaconyl-CoA decarboxylase and enoyl-CoA hydratase. Core metabolic functions such as glycolysis, the pentose phosphate pathway and pyruvate metabolism were shared across the major phyla. Acetate and propionate synthesis was found to be carried out by both the *Bacteroidetes* (*Bacteroides* and *Prevotella*) and *Firmicutes* and to a lesser extent the *Proteobacteria* and *Actinobacteria*. Cross-feeding between bacterial species has been observed, for instance, the *Bifidobacteria* have been found to cross-feed acetate as a product of inulin metabolism to butyrate producers (O’Callaghan & van Sinderen, 2016) and the fermentation of rhamnose by *Blautia spp* produces 1,2-propanediol that is used by *Eubacterium hallii* (Reichardt *et al*, 2018), illustrating the interdependent nature of the gut flora.

43 Blakeley-Ruiz *et al* (2019) also used metaproteomics to probe the functions of the gut microbiota, in this case that of five patients in remission from Crohn’s disease (3 females, 2 males aged 52 to 75 years). Catabolic functions of the microbiota, like the metabolism of N-acetylglucosamine and acetylneuraminate from host-ingested food and the production of SCFAs that have functions in the host, appeared to be shared across the major phyla in a dynamic way such that despite changes in taxonomic composition, overall functions remained. While acetate production was ubiquitous, the enzymes for propionate and especially butyrate synthesis were not found in all 5 individuals, which agreed with historical observations that SCFAs, butyrate in particular, were reduced in Crohn’s disease patients.

44 Eng & Borenstein (2018) investigated the property of taxa-function robustness in the microbial communities in samples taken from different body sites that were part of the Human Microbiome Project. Taxa-function robustness was defined as the degree to which a shift in a community’s taxonomic composition will affect its functional capacities. Bacterial communities in the digestive tract in general were much more functionally stable, despite taxonomic shifts, than those from other body sites such as the skin, nostrils and vagina.

45 Gutierrez and Garrido (2019) created pan-bacterial consortia from known gut microbiota (five *Firmicutes*, seven *Bacteroidetes*, one *Proteobacterium* (*E. coli*) and one *Actinobacterium* (*Bifidobacterium longum*)) and in each case, omitted one species and observed the effect that the omission had on the growth of the rest. Inulin was the carbon source. Bacterial growth, colony composition, acetate, propionate, butyrate and lactate production were measured. Deletion of *E.coli* or *Bacillus thetaiotaomicron* resulted in increased growth whereas deletion of *Bacteroides dorei* or *Lachnoclostridium clostridioforme* reduced the growth rate, indicating that different species can have negative as well as positive effects on the community. *Bacteroides fragilis* appeared to interfere with inulin metabolism since more of the substrate remained at the end of the incubation period in its presence than in its absence. The negative effect of *E.coli* was also seen on the growth of the

prominent inulin utiliser *Bifidobacterium adolescentis*, which increased its relative abundance to about 60% in the absence of *E.coli*. SCFAs increased or decreased depending upon the consortium composition, with various species responsible for the production of different acids and downstream effects on other species. Thus, although there is functional redundancy, there are also keystone taxa that influence overall diversity and growth. The authors suggested that such studies could be used to design microbial consortia with desired metabolic features.

46 Despite the above results illustrating the stability of the functions of the gut microbiota, Bradley and Pollard (2017) found that the expression of some genes showed significant variability between individuals and that many metabolic pathways had at least one variable element. The presence of elevated titres of *Proteobacteria* and to a lesser extent the archaeal phylum *Euryarchaeota* were correlated with the expression of variable genes while, although there were taxonomic shifts in the *Firmicutes* and *Bacteroidetes*, they did not seem to be major sources of variable genes. Since the *Proteobacteria* had previously been correlated with increased risk of metabolic syndrome and IBD, the authors suggested that the genes could be used as biomarkers for these conditions.

47 In the light of methodological trends in her lab and others, Theriot (2018) speculated that "...the systems microbiology field is going to be focused on targeted engineering and editing of the microbiome to alter function, which will be leveraged to prevent and/or treat human diseases..." and "... newer model systems...such as organoids and bioreactors, will be advantageous in dissecting microbiome function."

Intestinal barrier

48 The digestive tract has an enormous internal surface area and is a major interface with the external environment. It functions as the site of nutrient uptake into the body, while at the same time it has to protect the organism from extraneous chemicals and microorganisms. Potentially pathogenic organisms are ingested with food, so it is vitally important for the health of the host that these pathogens are controlled and do not cause disease. The digestive tract of humans and other animals has evolved a number of structures and functions that act to prevent invasion by pathogens, and these are collectively termed the intestinal barrier. Synonyms such as gut, colonic, or colonisation barrier are also used in the literature. There are a number of recent reviews concerning the intestinal barrier and the role of the microbiota in its maintenance. For example, Takishi *et al* (2017), Shi *et al* (2017), Walker (2014), Camilleri *et al* (2017).

49 Far from being a passive physical barrier, the gut epithelium has a dynamic and responsive system to maintain its integrity and different functions operate depending upon their proximity to the cellular layer. In the lumen, in addition to degradation of invading bacteria and antigens by gastric and pancreatic secretions as they pass through the gut, commensal bacteria produce antimicrobial substances. The microclimate close to the epithelium (unstirred water layer, glycocalyx (a meshwork of carbohydrate moieties of glycolipids or glycoproteins, including transmembrane mucins), and the mucus layer that is produced by epithelial goblet

cells) prevents bacterial adhesion and contains antimicrobial products secreted by Paneth cells (including α -defensins and the lectin RegIII) and secretory IgA from enterocytes. Below the unstirred water layer, glycocalyx, and mucus layer, the epithelial cells are separated by tight junction proteins that inhibit physical passage through the cell layer.

50 Interaction of bacterial surface proteins with Toll-like receptors (TLRs) on epithelial and immune cells leads to cytokine release that promotes the differentiation of T-helper cells and maintains gut innate immune homeostasis (Okumura *et al* (2017)). The development of the commensal gut microbial community in infancy appears to lead to T-cell tolerance of these organisms and helps B cells mature into IgA producing cells in structures such as Peyer's Patches².

51 As animals and people age, the protective immune functions of the gut appear to decline, leading to an increase in inflammatory cytokines and breakdown of the epithelial tight junctions, causing increased permeability (leaky gut). Bacterial populations change, with an increase in LPS producing species and a decrease in *Firmicutes* species (reviewed by Takiishi *et al*, 2017).

52 Loke and Lim (2016) highlight a differential innate immune response in mice as a result of colonisation with the unicellular protozoan *Triticomonas muscule* (*T. mu*), which increases bacterial resistance at the expense of increased inflammation, and helminths, which appear to stimulate opposite responses. Although there has been no equivalent finding in humans and the immunology of these organisms is still poorly described, it points to the potential for different classes of organisms to interact with each other and lead to changes in the immune balance of the gut and effects on host health. The changes produced may be neither entirely detrimental nor entirely beneficial.

Effect of xenobiotics on the gut microbiota

53 The majority of experiments in the literature pertaining to the effects of xenobiotics on the gut microbiota have been performed in animals, mostly in mice. A brief description of recent papers is given in Appendix 4 in Tables 1 (metals), 2 (pesticides), 3 (antibiotics) and 4 (miscellaneous). While changes on rodent gut flora may not relate directly to the changes that may take place in humans, they indicate the type of effect that might be caused, which may not be possible or ethical to show with humans. The findings are briefly summarised below:

² Peyer's patches are lymphoid tissue involved in the maturation of IgA-producing B cells and are widely distributed in the small intestine and to a lesser extent in the colon. These patches contribute to generating B cells and plasma cells. Activated B cells in Peyer's patches consistently generate IgA-producing plasma cells for T cell-dependent and T cell-independent responses in the gut. Secretion of IgA is their major contribution to protecting the gut barrier.

54 In the animal studies, xenobiotics in all four groups, either gavage dosed or administered in the diet or drinking water, could affect the balance of the bacterial phyla in the gut. Heavy metals such as arsenic, cadmium and lead tended to reduce the *F/B* ratio and lead to reduced SCFA production and increased oxidative stress. Iron, copper and nanoparticulate titanium, silver and gold tended to increase the *F/B* ratio but also reduced SCFA production. All of the metals tested affected various bacterial genera to different degrees.

55 Organophosphate insecticides (chlorpyrifos, diazinon and malathion) and the carbamate aldicarb, which all exert their intended activity via acetylcholinesterase inhibition, were not found to have any single consistent effect on the microbiota but all affected the taxonomic balance to certain degrees. Different studies found an increase in oxidative stress, greater expression of virulence genes and effects on host lipid metabolism. Glyphosate also affected the taxonomic balance but, in one study, this effect was found to be reduced in the presence of pre-formed aromatic amino acids in the gut.

56 Oral antibiotics (lactams and non-lactams) changed the balance of microbiota by decreasing some taxa but also by increasing others. In some cases, resistance to the antibiotic used increased. The study of Zhang *et al* (2013) indicated that knowledge of the pharmacokinetics of an antibiotic could be used to tailor how it should be used clinically to best leave the gut flora unaffected, i.e. if it is not excreted in bile then a parenteral route could be used.

57 A range of other compounds has been tested on the gut microbiota of rats and mice. This includes artificial sweeteners, mycotoxins, ethanol, dioxins, flame retardants, BPA and recently (Young *et al*, 2020), industrial methylimidazolium ionic liquids, for example 1-octyl, 3-methylimidazolium chloride. These studies found a variety of taxonomic changes, sometimes with increases in potentially inflammatory conditions and decreases in “beneficial” bacteria, although this was not always the case.

58 Wheeler *et al* (2016) tested the effects of the fungicide fluconazole on the gut mycobiota of mice and found changes in the titre of various species (*Candida spp* decreased, *Aspergillus spp* and others increased) that were concomitant with increased severity of dextran sodium sulfate (DSS)-induced colitis and house-dust-mite-extract-induced allergic airway disease.

59 The data from animal studies suggest that almost anything consumed may have the effect of changing the balance of the gut microbiota, sometimes in what would appear to be a detrimental direction, albeit the relevance of the dose used was sometimes questionable. This might indicate a potentially increased risk of the growth of pathogens, reduction in both the barrier function and the health of the gut epithelium and thence may lead to systemic effects on the host. However, results vary between studies: the parameters chosen to be observed are not the same in all cases, conditions differ between studies and there is little indication as to whether changes are adverse or adaptive. Despite the often unphysiologically high concentrations of xenobiotics used in animal experiments in order to elicit a response, there is also widespread extrapolation of results to the human condition.

Human *in vivo* and *in vitro* studies

Metals

60 Dong *et al* (2017) investigated the effects of arsenic (As) in drinking water on the intestinal microbiota of Bangladeshi children. High arsenic concentrations ($219 \pm 166 \mu\text{g/l}$) correlated with a relative enrichment of bacteria in the *Proteobacteria* phylum ($p < 0.03$) without statistically significant effects on the *Bacteroidetes*, *Firmicutes* or *Actinobacteria*. Of 322 genes that showed increased expression in the presence of high arsenic, 78% (258) were found to be in antibiotic resistant bacteria. The *E. coli* genes associated with arsenic resistance that were seen in Bangladeshi children were not seen in children in a European cohort, where drinking water As levels are lower, suggesting that the Bangladeshi bacteria had adapted to the metal.

61 Calatayud *et al* (2018) used a SHIME (Simulator of the Human Intestinal Microbial Ecosystem) *in vitro* model system of the human gut microbiota to investigate the role of salivary and gut microbiota in the bioaccessibility, biotransformation and intestinal absorption of arsenic from different foodstuffs: mussels, seaweed and rice. Colonic conditions were simulated by adding donated faecal samples to a nitrogen-flushed bioreactor. Caco-2 cell monolayers were incubated with the digestion products. No arsenic appeared to be transported into the blood-resembling matrix, but bacteria-conditioned digestion of the food material led to 1.4 – 2.8-times greater cellular uptake compared with non-digested food.

62 Yin *et al* (2019) reported the production of nanoparticles from silver in a SHIME model. Faecal samples were collected from two healthy volunteers and incubated in a solution containing 1 mM silver nitrate at 37°C for 48 hours. Spherical silver nanoparticles were observed under transmission electron microscopy on the surface of and within microbial cells. The authors expressed concern that nanoparticles could be produced by gut microbiota, with unknown consequences for microbial and host health.

63 Cattò *et al* (2019) studied the interactions between non-lethal concentrations of citrate-capped silver nanoparticles (AgNP) (1 $\mu\text{g/ml}$ final concentration), human intestinal microbiota and a probiotic organism (*Bacillus subtilis*, BS, 10^7 cells/ml) in an *in vitro* batch incubation model. After 24 hours incubation, all conditions (control, AgNP, BS alone and AgNP-BS) led to a depletion of the *Bacteroidetes* with an increase in the *Firmicutes* and *Proteobacteria*. The other treatments all slightly raised the *Bacteroidetes* level, with treatment with BS alone having the greatest effect. AgNP and AgNP-BS treatment led to a 56% increase in the level of the *Megasphaera* genus, which has been associated with antibiotic resistance and stress response. At the species level, decreases were noted in the titres of *Faecalibacterium prausnitzii* and *Clostridium coccoides*/*Eubacterium rectales*, a finding seen in patients with intestinal inflammation and ulcerative colitis. No treatment affected SCFA production, but AgNP-BS markedly counteracted functional changes induced by treatment with AgNP alone, particularly in the microbiota's capacity for xenobiotic degradation and metabolism.

64 Meyer *et al* (2008) reviewed papers showing that methanoarchaea isolated from the human gut (*Methanosphaera stadtmanae*, *Methanobrevibacter smithii*)

exhibit a high potential for the derivatisation of metal and metalloid (arsenic, antimony, bismuth, selenium, tellurium and mercury) into toxic volatile products compared to bacterial gut isolates, and assumed that the methanoarchaea in the human gut are mainly responsible for the production of these toxic volatile derivatives. One of these, trimethylbismuth ((CH₃)₃Bi), the main volatile derivative of bismuth produced in human faeces, was found to inhibit the growth of cultures of *Bacteroides thetaiotaomicron*, a representative member of the human physiological gut flora, suggesting that these organic derivatives may be toxic to human health both through direct interaction with host cells and by disturbing the gut microflora.

Pesticides

65 Reygner, Condette *et al* (2016) used the SHIME model to study the direct effects of below-threshold chlorpyrifos (1 mg/day) on the composition, diversity and metabolic functions of the human gut microbiota. Changes in the measured parameters were observed in the different compartments of the model but how they were observed depended upon the method used: PCR found no significant change in overall titre over 30 days whereas culturing found a significant increase in both aerobes and anaerobes. Cultured *Bacteroides* spp and *Clostridia* spp increased but *Bifidobacterium* spp decreased in the colon reactor. Of the SCFAs, changes were temporary increases or decreases in different compartments at different times. The authors suggested that although the changes measured were modest, they might have an impact that could affect health in very young infants.

66 Schneeberger *et al* (2018) treated hookworm-positive adolescents aged 15 to 18 years from Cote d'Ivoire with four anthelmintic drug regimens (tribendimidine alone, tribendimidine + ivermectin, tribendimidine + oxantel pamoate and albendazole + oxantel pamoate) for three weeks. The gut bacterial composition was analysed using 16S rRNA gene sequencing. On treatment arm 2 (tribendimidine + ivermectin), the titre of *Bacteroidetes* in stool samples was increased due predominantly to the abundance of the families *Prevotellaceae* and *Candidatus homeothermaceae* at 24 hours after treatment. This effect was transient and disappeared three weeks after treatment. Increases were noted in biotin metabolism, folate synthesis and N-glycan biosynthesis. No changes were observed following the other treatments. The authors recommended further study of this drug interaction.

Antibiotics

67 Antibiotics alter the structure of the human gut microbiota. At least 42 genera have been found to be sensitive to the effects of a range of 68 different antibiotics. However, the effects of antibiotics are difficult to ascertain with the commonly used 16S rRNA genetic analysis since this technique detects all bacteria in a population, including those that are dead or quiescent. Bacteria in the gut that are susceptible to antibiotics are replaced by others that fulfil the same functions but are resistant to treatment, but this can also lead to an imbalance of function causing detrimental effects on the host such as greater risk of obesity and/or type 2 diabetes. (Review by Ferrer *et al* (2017)).

68 Isaac *et al* (2017) administered the antibiotic vancomycin to rheumatoid arthritis patients at 250 mg four times a day for 2 weeks followed by methotrexate for 6 weeks. A control group received methotrexate only from the beginning of the study. Vancomycin reduced the richness and diversity of the human microbiota samples, vastly reducing the levels of the *Bacteroidetes* with a slight increase in the *Firmicutes* (driven by the genera *Megasphaera* and *Veillonella*) and large increases in the *Proteobacteria* and the *Fusobacteria*. Recovery from antibiotic treatment varied between individuals. The rate of recovery in a mouse model was negatively associated with infection with pathogens such as *Klebsiella*, *Escherichia* and *Shigella*.

69 Of 102 patients treated with antibiotics, Meletiadiis *et al* (2017) observed amplification of an AMR gene in 20/56 (36%) patients treated with ceftriaxone alone or in combination (3/20 with ceftriaxone alone, 17/36 with ceftriaxone and another antibiotic) and 10/46 (22%) with other antibiotics (6/10 treated with ciprofloxacin or levofloxacin with other antibiotics). No AMR gene amplification was seen in control patients. The authors recognised that only the amplification of the beta-lactamase *bla*_{CfxA-6} gene was explored, which left open the question of what other genes may have been amplified by treatment and the full extent of the effect of the antibiotic.

70 Raymond *et al* (2016) treated eighteen healthy volunteers twice a day for 7 days with an oral dose of 500 mg cefprozil, a second-generation cephalosporin, and the participants collected faeces samples at three time points: before the antibiotic treatment at the end of the treatment, and 90 days after the end of the treatment. Six non-treated volunteers acted as controls. Metagenomic DNA sequencing was performed on the faecal samples. Each participant had a specific subset of *Bacteroides* species and inter-individual variability at the species level was greater than the effect of the antibiotic in most cases. Species belonging to the genera *Akkermansia*, *Alistipes*, *Bacteroides*, *Dialister*, *Parabacteroides* or *Prevotella* were typically found, with *Bacteroides* being dominant in 40 out of 72 samples. The most consistent effect of the antibiotic was the increase of *Lachnoclostridium bolteae* in 16 out of the 18 cefprozil-treated subjects. A subgroup of participants was found to be enriched in the opportunistic pathogen *Enterobacter cloacae* after antibiotic treatment, an effect linked to lower initial microbiome diversity and to a *Bacteroides* enterotype (a bacterial population enriched in the named taxon), but levels had returned to pre-treatment levels by 90 days post-treatment. The AMR gene content of participants' microbiomes was found to be altered by the cefprozil in a manner specific to the individual. Point mutations in beta-lactamase *bla*_{CfxA-6} were enriched after antibiotic treatment in several participants. The authors suggested that monitoring the initial composition of the microbiome before treatment could assist in the prevention of some of the adverse effects associated with antibiotics or other treatments.

71 Maurice *et al* (2013) took faecal samples from three healthy adult volunteers and characterised their bacterial populations *ex vivo* in the presence and absence of antibiotics and host-targeted drugs. Bacteria were typed into groupings of low or high nucleic acid content (LNA and HNA, respectively), which corresponded with their metabolic activity, and types that were measured as damaged, either by loss of membrane integrity (Pi*) or of membrane polarity (DiBAC⁺). The HNA/ high energy phenotype correlated with the highest level of damage and was due to the

Clostridiales within the *Firmicutes* phylum. The *Bacteroidetes*, in particular the *Bifidobacteriales*, were low energy/ LNA organisms. Upon antibiotic treatment, especially with cell wall-targeting compounds, the proportion of damaged cells increased, without changes in the proportion of HNA/LNA, suggesting membrane damage without complete lysis. The *Firmicutes*, being Gram-positive, were particularly affected. There was substantial temporal variation in damage to the structure of the microbial community following antibiotic treatment, although this was small when compared with inter-individual differences. By contrast, host-targeting drugs produced only very minor changes in community structure. A variety of genes were up-regulated in response to antibiotic and non-antibiotic drug treatment.

72 Arat *et al* (2015) treated 61 healthy volunteers in a dose-escalation study of GSK1322322, a novel antibiotic targeting the bacterial enzyme peptide deformylase, with iv-only and oral-and iv dosing. Only the oral-and -iv regimen affected the balance of bacterial taxa in the volunteers' faeces samples, with decreases in the titre of species in the *Bacteroidetes* and *Firmicutes* and an increase in members of the *Actinobacteria* and *Protobacteria* at the end of the study. Functionally, there was an overall decrease in metabolic pathways for terpenoids and polyphenols, protein folding, sorting and degradation and the metabolism of cofactors and vitamins. Functions increased included multi-drug transporters, xenobiotic metabolism and signal transduction. The authors pointed out that this was the first human study to highlight the difference in the effect of oral vs iv dosing on the gut biota and pointed out the similarities in their results to the mouse study of Zhang *et al* (2013, Table 3).

73 De Gunzburg *et al* (2018) treated 44 healthy volunteers orally for 7 days with moxifloxacin (MFX, 400 mg, n = 14), 400 mg MFX-plus-DAV132 (a form of activated charcoal, 7.5g) n = 14, DAV132 alone (5g n=8) or a control consisting of the DAV132 preparation with microcrystalline cellulose instead of activated charcoal (n=8). Faecal and blood levels of MFX were measured over the treatment period. DAV132 treatment had no significant effect on the blood pharmacokinetics of MFX but significantly reduced the MFX faecal AUC. The MFX associated reduction in microbiota and faecal gene richness did not occur in the presence of DAV132. DAV132 also absorbed a range of other antibiotics in pig caecal material *in vitro* (penicillins, first and third generation cephalosporins, carbopenems, fluoroquinolones and the lincosamide clindamycin, all at 400 µg/ml). The authors suggested that their DAV132 preparation may be co-administered to protect the human gut microbiome against the deleterious effects of many antibiotics.

74 Nogacka *et al* (2017) studied the effects of maternal antibiotic prophylaxis during the intrapartum period on the intestinal microbiota and the presence of a range of AMR genes in vaginally delivered neonates. Of 40 mothers who had uncomplicated vaginal deliveries, 12 with confirmed or suspected vaginal group B streptococcal infection were treated with 5 million units of a penicillin while giving birth, followed by 2.5 million units every four hours until delivery was complete (intrapartum antibiotic prophylaxis, IAP). The other 22 mothers without infection did not receive an antibiotic (control). Infants were fed either breast milk or formula. Faecal samples were taken for analysis at 2, 10, 30 and 90 days of age. Changes in bacterial taxa were tested for by 16S rRNA gene sequencing and the presence of a range of lactam and non-lactam AMR genes by PCR.

75 In the above study, IAP caused a slight but non-significant delay in SCFA production as seen up to 10 days but thereafter there was no observed effect. The infants of IAP exposed mothers had a consistent, but non-significant, elevation of the *Protobacteria* across the time course and an increase in the *Firmicutes* that was significant when compared with non-antibiotic-treated controls at 10 and 90 days of age. The *Actinobacteria* and the *Bacteroidetes* were relatively increased in the controls and this became significant only at 10 days for the *Actinobacteria* (all significance levels $p < 0.05$). Of the AMR genes, none of the infants tested positive for *tetM*, *tetO*, *tetA* (tetracycline ribosomal protection protein, trpp), *strA* (aminoglycoside phosphotransferase) or *cmlA1* (chloramphenicol efflux pump). Of the others (*bla_{tem}*, *bla_{CTX-M}*, *bla_{SHV}* (β -lactamases), *mecA* (penicillin binding protein 2a), *tetW* (trpp) and *aac(6*)-le-aph(2*)* (aminoglycoside acetyltransferase/ phosphotransferase), tested positive in more than 30% of the infants. The *bla_{tem}*, *bla_{CTX-M}* and *aac(6*)-le-aph(2*)* were slightly elevated by IAP, the highest being *bla_{tem}* (82.3% positive samples in the IAP group vs 61.9% in the controls), but none reached significance. Overall, the authors concluded that their results indicated that maternal IAP induced a shift in the balance of the newborn taxa for at least 3 months after birth and an increase in β -lactamase expression that warranted further study.

76 Neuman *et al* (2018) reviewed the effects of pre-term and early life antibiotic treatment on the gut microbiota of children and possible later onset effects. Exposure took place in different studies (2005 – 2016) between the pre-natal period and up to 7 years of age, with evaluation of effects in some cases in the first days, weeks or months after birth and in others over several years. A variety of antibiotics was used including penicillins, aminoglycosides and macrolides. Changes were noted in gut microbial composition, abundance and diversity (often an increase in *Proteobacteria* and *F/B* ratio) and in the cases where such data were collected, treatment correlated with increased risk of overweight/obesity, asthma, IBD, food allergies and increased expression of AMR genes.

77 Sarmiento *et al* (2019) compared the presence of AMR genes in the human GI tract with xenobiotic intake and weight of 72 adult volunteers. Participants were between 18 and 60 years of age with no record of occupational disease, diabetes or recent antibiotic use. Food frequency questionnaires were completed over 6 months, body mass indices were measured, and faecal samples were taken. Twenty-seven markers for AMR genes were screened for by PCR, covering beta lactams, tetracyclines, aminoglycosides, quinolones, sulfonamides, and macrolides. A total of 17 AMR genes were found in the samples taken from normal, overweight and obese individuals, 2 were shared between normal and obese, 3 between overweight and obese and 4 and 1 were unique to the obese and overweight groups respectively. Overall expression of resistance increased with bodyweight, as did the density of faecal bacteria in Gram positive cocci, and aerobic and anaerobic Gram negative rods. At the genus level, the greatest increases were in the *Escherichia* and the *Enterococci*.

Miscellaneous

78 Zhang *et al* (2018) exposed 4 species of common gut bacteria (*E. coli*, *Bacteroides fragilis*, *Clostridium sporogenes* and *Streptococcus gallolyticus*) to a

cocktail of 29 xenobiotics (plasticisers, colorants, flame retardants and personal care products) at a final concentration of 1mM/compound. Compounds included bisphenol A, rhodamine B, triphenylphosphate and triclosan. The cocktail was added to growth media to give an exposure concentration of 10 or 100 nM for each compound, to mimic the range of human exposures. Minimal effects were seen on bacterial growth and morphology over 48 hours. Multiple changes in metabolite profiles were observed at the 100 nM level, affecting sulfur-containing amino acids, putative neurotransmitters, intermediates of energy metabolism and oxidative stress products. Responses differed between bacterial species and between species sharing the same growth habit, eg anaerobiosis. In addition, the authors pointed out that the involvement of secondary metabolites had not been addressed and would require a more comprehensive study.

Montassier *et al* (2017) investigated the effect of chemotherapy with a cocktail of agents (bis-chloroethylnitrourea, etoposide, aracytine and melphalan) on the gut microbiota of 28 patients with non-Hodgkin's lymphoma. There was a reduction in the abundance of the *Firmicutes* ($p=0.0002$) and the *Actinobacteria* ($p=0.002$) and increases in the *Proteobacteria* ($p=0.0002$) after 7 days' treatment. Functional shifts were noted, with reductions in amino acid, nucleotide and energy metabolism but increased inflammation-related signal transduction and glycan metabolism. A decrease in butyrate-producing bacteria suggested that the thickness of the epithelial mucus layer may have been reduced and the authors speculated on the influence of the microbiota in the gastrointestinal toxicity seen during chemotherapy.

79 Hill-Burns *et al* (2017) observed changes in the gut microbiota of Parkinson's Disease (PD) patients that appeared to be brought about by the disease itself and the type of drug used in its treatment. Stool samples from 197 PD and 130 control patients were analysed. PD patients were also analysed for medication-induced changes. Disease duration was correlated with an increased abundance of *Ruminococcaceae* ($p = 0.0005$). Earlier reports had found that PD was associated with increased abundance of *Akkermansia*, *Lactobacillus* and *Bifidobacterium*, with reduced levels of *Lachnospiraceae*. This study found that catechol-O-methyltransferase (COMT) inhibitors and anticholinergic drugs reduced *Bifidobacterium* levels and increased the *Lachnospiraceae*. PD-induced depletion in the latter family is consistent with a decrease in SCFA production, and consequent adverse effects on host physiology. The authors speculated that the initial lesion in PD may be in the gut, which then has downstream effects in the central nervous system.

80 Minalyan *et al* (2017) reviewed the effects of proton pump inhibitors (PPI) on the gastric and intestinal human microbiome and highlighted the work of Jackson *et al* (2016), who followed changes in the gut microbiome of 1827 pairs of healthy twins in the presence and absence of PPI use in relation to other confounding factors such as BMI, frailty and diet. Abundance of the gut microbiota decreased in the presence of PPI use, but this was not significant when other factors were considered. However, PPI use appeared to lead to a greater proportion of oral/pharyngeal taxa entering the gut, especially the *Streptococcaceae* and the *Micrococcaceae*. The changes observed were independent of antibiotic use although this was not associated with individual antibiotics and use was not prolonged. The authors suggested that the relative abundance of the *Streptococcaceae* was of clinical

importance since small intestinal bacterial overgrowth of these organisms is known to be associated with *Clostridium difficile* infection.

81 Maier *et al* (2018) measured the growth of 40 faecal bacterial isolates after treatment with 1197 compounds covering human-targeted drugs, antibiotics, antiseptics and others with antifungal, antiviral and antiparasitic actions. Bacterial growth was measured by optical density in multiwell plates under anaerobic conditions. All drugs were at 20 μ M final concentration. Of 156 antibacterials, 78% were active against at least one species and 27% of the non-antibiotic drugs were also found to be active, including 40 that were effective against ten bacterial strains, of which 14 drugs had no previously documented antibacterial activity. Species with higher abundance across healthy individuals, including major butyrate and propionate producers, were significantly more affected by human-targeted drugs than others. Moreover, the authors estimated that the concentration of the drugs used would be lower than that actually encountered in the small intestine and colon under clinical dosing, leaving open the questions of which other drugs might be active at higher concentrations and how many taxa might be affected. The authors also found that exposure to human-targeted drugs can promote non-specific resistance mechanisms such as efflux transporters, which can contribute to antibiotic resistance.

82 Shanahan *et al* (2017) sampled the duodenal mucosa-associated microbiota (MAM) of 102 Australian hospital patients, who were sub-grouped into current smokers (n = 21), previous smokers (n = 40) and never smokers (n = 41). Recent antibiotic use was an exclusion factor but functional dyspepsia (FD), iron deficiency (ID) and Crohn's disease (CD) were not. Overall, smoking did not appear to affect the bacterial load of the mucosa but both current and previous smokers had significantly reduced bacterial diversity compared with never smokers. When broken down further, CD sufferers were less affected than FD/ID sufferers. 16S rRNA gene sequencing revealed that in current smokers, the *Firmicutes* were significantly increased and the *Bacteroidetes* and *Actinobacteria* significantly reduced relative to the never smokers (p < 0.005). At the genus level, *Streptococcus*, *Rothia* and *Veillonella* were increased in current smokers relative to never smokers. Reduction in duodenal oxygen tension and pH were suggested as potential drivers of the effects seen and the authors highlighted the possibility that smoking could be regarded as another confounding factor of experiments on the gut microbiota.

The effect of food components on the gut microbiota.

83 Roca-Saavedra *et al* (2018) reviewed the effect of minor food components on the gut microbiota and vice versa. For example, polyphenols found in a variety of fruit, vegetables and beverages both alter community composition via their antimicrobial actions and are metabolised to products with increased bioavailability. Other plant-derived compounds, such as flavonols, tannins and resveratrol, promote some taxa and inhibit the growth of others and are metabolised in ways that may affect their reported effects on host health.

84 Maier *et al* (2017) fed human adults (26 women and 13 men) with reduced insulin sensitivity high or low carbohydrate diets and supplemented groups of them with high-resistance starch (HRS, high amylose corn starch) or low-resistance starch (LRS, high amylopectin corn starch) in a cross-over design study with 2 weeks on each diet with a 2-week washout period between diets. Faecal and blood samples were taken before and after each diet-consumption period. The gut microbiota were affected most by the low carbohydrate diet with HRS. The HRS appeared to improve meal-to-meal regulation of blood glucose but also led to higher plasma levels of trimethylamine-N-oxide, which has been linked to increased risk of cardiovascular disease. In addition, HRS was found to increase the *Firmicutes/ Bacteroidetes* ratio and increased the numbers of butyrate- and propionate-producing genera. Genes related to lipid metabolism were variously up- or down-regulated by the HRS diet.

85 Costantini *et al* (2017) reviewed the effects of consuming omega-3-fatty acids on the structure and function of the gut microbiota. Results of the few (9) clinical studies reported were variable with the major phyla unaffected, increasing or decreasing in different studies. In some cases, potentially pathogenic genera were reduced in comparison to beneficial ones. Oils differed in being derived from plants in some cases and fish in others. In animals, omega-3-fatty acid deprivation resulted in anxiety and depression-like behaviour, increased activity in the hypothalamic-pituitary-adrenal (HPA) axis and gut inflammation along with *Firmicutes/ Bacteroidetes* imbalance. All of these conditions were improved by supplementation. The authors concluded from their review that omega-3-fatty acids were beneficial for gut microbiota, leading to greater epithelial integrity and function of the immune system and thence of the gut-brain axis.

86 Shinohara *et al* (2010) observed that apple pectin consumption was associated with an improved intestinal environment because isolates of “beneficial” bacteria such as *Bifidobacteria* and *Lactobacillus* from faecal samples from healthy human individuals were capable of metabolising this carbon source, whereas other, potentially harmful species such as *Escherichia coli* and *Clostridium perfringens* were not. Sahasrabudhe *et al* (2018) also observed that lemon pectins with various levels of methyl esterification ameliorated doxorubicin-induced ileitis in mice via activation of Toll-like receptor 2-1 but this effect did not appear to be mediated via microbial SCFA production. The authors concluded that the microbiota may not always be involved in the intestinal effects of xenobiotics.

87 Chassaing *et al* (2017) used the SHIME system to investigate the effect of the emulsifiers carboxymethyl cellulose and polysorbate 80, commonly used food additives, on the human microbiota. They then implanted the treated or control human faeces from the SHIME apparatus into gnotobiotic mice to observe any physiological effects. The emulsifiers were found to affect the bacterial diversity of the SHIME model and increased the level of inflammatory markers such as flagellin and LPS, but did not have marked effects on the gut of the mice when administered directly. However, proinflammatory changes were observed in the gut of mice into which the treated SHIME faeces was transplanted, suggesting that the initial effect of the emulsifiers was on the microbiota, which then affected the response of the host. The effects on the microbiota and the host were found to persist even after the emulsifiers would have been flushed from the gut, indicating that the changes in the host fed back on the microbiota, maintaining its altered phenotype.

88 Engen *et al* (2015) reviewed the effects of ethanol on the human gut microbiota. The studies reviewed showed that alcoholic patients with or without cirrhosis of the liver had increases in the *Proteobacteria* phylum, with various genera in the *Bacteroidetes* and *Firmicutes* either increasing or decreasing. The type of alcoholic beverage appeared to affect the ensuing changes, with some of the changes apparently induced in healthy people consuming red wine being beneficial in character, especially an increase in *Bifidobacteria*, that were not seen in consumers of gin. The difference between de-alcoholised red wine and gin were more marked, suggesting that the polyphenol content of the wine may have been responsible. The authors concluded that alcohol-disrupted intestinal barrier function, in combination with alcohol induced bacterial overgrowth and dysbiosis, could be relevant for the development of alcohol-induced liver pathology, including alcoholic liver disease (ALD). Studies showed that alcohol consumption disrupted the intestinal barrier via increased oxidative stress in the intestine, which in turn disrupted tight junctions and promoted intestinal hyperpermeability

89 Kosnicki *et al* (2019) compared the effects of ethanol consumption on the microbiota of Wistar rats, gavaged with ethanol at 20% v/v for 12 weeks with that of human “moderate” drinkers from the American Gut Project (AGP). Despite the fact that the consumption conditions differed markedly between the two studied groups, the authors felt that the large numbers of people in the AGP cohort were sufficient for patterns to become evident. The more acute exposure in the rats led to reduced alpha (within group) and beta (between treated and control group) microbial diversity in the treated group, with the balance of taxa tending toward an inflammatory phenotype. In the human group, alpha and beta diversity was significantly higher in the alcohol drinkers. Of particular note, however, was the downward trend of the genus *Lactobacillus* in both rats and humans. *Lactobacillus* is known to protect the gut epithelium from tight junction disruption and translocation of bacteria and LPS by metabolising acetaldehyde to acetate. There were some hints at a “rebound” in diversity towards the end of the rat study, suggesting that the responses of the rat and human microbiota might be more similar over longer timescales.

Sweeteners

90 Wang *et al* (2018) studied the bacteriostatic effects of 4 non-nutritive sweeteners (sucralose, saccharin, acesulfame-K and rebaudioside A (from Stevia)) on *E. coli* *in vitro* and on the microbiota, food intake and body weight of mice. Acesulfame-K and saccharin (0.25% w/v) exerted a bacteriostatic effect on two *E. coli* strains in liquid culture, as measured by OD₆₀₀, whereas sucralose did not. Rebaudioside (2.5% w/v) inhibited the growth of *E. coli* strain HB101 on agar, but not that of strain K-12. *In vivo*, a high fat diet in mice increased food (and hence calorie) intake and reduced water intake and sucralose had no effect on this, but high fat also reduced faecal output and sucralose partially reversed this effect (p<0.05). On normal diet, mice fed sucralose showed a significant increase in the *Firmicutes* (p<0.05) and a trend to reduced *Bacteroidetes* (p = 0.117), without changes in the other major phyla. At the genus level, sucralose significantly increased the abundance of the *Bifidobacteria* but not the *Clostridia*. Despite the sometimes higher than dietary levels of some of the sweeteners used, the authors concluded that non-

nutritive sweeteners exert a variety of effects on the microbiota with consequences for the host that should be followed up.

91 Lobach *et al* (2019) reviewed the area of low/ no-calorie sweeteners on the gut microbiota. Papers on acesulfame-K, aspartame, cyclamate, saccharin, neotame, sucralose and rebaudioside A were discussed. Changes in the balance of the microbiota are noted in some studies but not in others and different studies highlight different bacterial genera. Most studies used doses higher than the Acceptable Daily Intake (ADI) and could not therefore be equated with the dose of these compounds as generally used by humans. The review reported that metabolic studies in mice, rats, and humans have shown that sucralose is largely unabsorbed by the gut but is not a substrate for gut microbiota. No change in the metabolic profile was seen after a 1-year exposure, indicating no microbial metabolic adaptation, even with very high doses. Neither saccharin nor acesulfame K underwent gastrointestinal metabolism, but, in contrast to sucralose, both of these sweeteners were rapidly absorbed and excreted unchanged in the urine. Steviol glycosides passed unabsorbed through the upper portion of the gastrointestinal tract but in the colon the sugar moieties attached to the steviol backbone were removed by the gut microbiota, primarily of the *Bacteroidaceae* family. Steviol was not a substrate for the intestinal microbiota and was absorbed from the colon intact. Following absorption, it was conjugated with glucuronic acid, and primarily excreted in humans as steviol glucuronide via the urine. The authors concluded that considering the extensive safety databases that have evolved over the years for these structurally unrelated sweeteners, there was little in the available studies of the microbiome to suggest that they raised safety concerns at their currently applied levels.

Environmental Pollutants and Contaminants

92 The environment contains a rich variety of chemical entities that may enter the digestive tract and have toxicological consequences either directly or by affecting the composition and/ or functioning of commensal organisms.

93 Claus *et al* (2016) reviewed the involvement of the gut microbiota in the toxicity of environmental chemicals. For example, the microbiota are capable of oxidising the widespread environmental pollutant benzo[a]pyrene (BaP) to its oestrogenic 7-hydroxy derivative and can deconjugate the hepatic product of phase 2 metabolism of BaP back to the parent compound. PCBs can be methylsulfonated by gut bacteria to products that are implicated in lung dysfunction, azo dyes such as Sudan 1 can be cleaved into potentially carcinogenic aromatic amines and melamine can be oxidatively deaminated to cyanuric acid, which may cause nephrotoxicity. This is in addition to the development of dysbiosis induced by the toxicity of the ingested chemical.

94 Snedeker and Hay (2012) reviewed evidence for the contribution of gut microbiota and environmental chemicals in the development of obesity and diabetes. They pointed out the associations that have been reported between gut dysbiosis in obese and diabetic individuals and the possible correlation between exposure to various pollutants, such as heavy metals, insecticides, and putative oestrogenic

chemicals and suggested that the interaction was a subject to which resources should be applied.

95 Saint-Cyr *et al* (2013) assessed the effects of No-Observed-Adverse Effect-Level doses of the mycotoxin deoxynivalenol (DON) on human gut flora transplanted into germ-free male Sprague Dawley rats. The NOAEL established for DON was 100 µg/kg bw per day, based on a decrease body weight gain reported in a 2-year feeding study in mice. After allowing the faecal transplants 2 weeks to stabilise, rats were administered DON at 100 µg/kg bw per day by gavage for 4 weeks. Faecal samples were collected weekly up to day 27 and then on day 37, 10 days after stopping treatment, and frozen until analysis. DON exposure increased the titre of the *Bacteroides/Prevotella* group of organisms during dosing ($p < 0.01$), but this declined to control levels before the end of the experiment. The *Bifidobacteria*, *Clostridium leptum* group and the *Lactobacillus/Leuconostoc/Pedococcus* group were unaffected by DON but *E.coli* was significantly reduced ($p < 0.01$) and this reduction persisted until the end of the experiment. The authors suggested that the investigation of the influence of low concentrations of mycotoxins on human gut microbiota should be a part of the risk assessment process.

96 Inhaled PM2.5 and PM10 particles from natural (e.g. forest fires, volcanoes) and man-made (e.g. vehicle exhausts, smoking) may be delivered to the digestive tract from the lungs by the action of the mucociliary escalator system in the trachea. (Salim *et al* 2013). This is, however, likely to be a minor route of GI exposure to xenobiotics.

97 Defois *et al* (2017) investigated the effect of BaP on the microbiota in samples of human faeces *in vitro*. Samples from two donors were incubated with BaP in sunflower oil at 0.005, 0.05, and 0.5 mg/l. The composition of the microbiota was determined by 16S-rRNA gene sequencing and a range of volatile compounds produced by the bacteria (the “volatolome”) was assayed using solid-phase extraction coupled with GC-MS. No significant changes were observed in the microbiota at the phylum level with only minor changes at the family level as a result of BaP treatment, but the baseline composition differed between the two donors. Seven volatile products were detected by the GC-MS system and tentatively identified from the literature of internal data, with a seventh of unknown identity. All detected compounds were significantly changed in level by at least 0.5 mg/l BaP with some (benzaldehyde and 2-methylphenol) showing significant increases and others (such as 2-hexylfuran and butylbutanoate) showing significant decreases. After 24 hours of incubation a wide range of metabolic pathway genes were upregulated by 0.5 mg/l BaP in both samples (21 in sample1, 31 in sample 2) but only 3 in each sample, different in each, were downregulated. The authors considered that the microbiota were adapting to the presence of the BaP and in doing so their change in metabolism could have down-stream effects on the gut and host health.

98 Defois *et al* (2018) studied the effect of a range of environmental pollutants (TCDD, PhIP, α and γ HBCDD, BaP, deltamethrin and a mixture of PAHs) on the gut microbiota from a human volunteer *in vitro*. The compounds were tested at 0.005, 0.90, 2.60, 5, 21 and 38 µg/l. Of the volatile microbial products assayed, 5, 2, 7 and 4 of them were significantly altered in concentration by deltamethrin, PhIP, TCDD

and the PAHs, respectively. These included ketones, xylenes and phenols. BaP and PAH mixture exposure in total up-regulated 613 genes and down-regulated 419 genes.

99 Gnotobiotic female C57BL/6 mice were used by Stedtfeld *et al* (2017) to study interactions between TCDD and segmented filamentous bacteria -SFB - (*Candidatus* Savagella) in the AhR-induced regulation of regulatory T cells (T_{reg}) in the gut. The mice were colonised by either *Bacteroides fragilis* as a representative commensal organism alone or in combination with the SFB and treated with either TCDD at 30 µg/kg bw or sesame oil vehicle every 4 days for 28 days by oral gavage. Other mice were treated with TCDD or vehicle in the presence of the SFB alone. In general, genes related to T cell differentiation were downregulated in response to TCDD and upregulated in response to SFB whereas the *B. fragilis* exerted a lesser effect. The effect of SFB on the response of T_{reg} was also seen in the spleen, blood and mesenteric lymph nodes. The authors suggested that continued work on the immune regulatory effects of the gut bacteria may lead to treatments for intestinal pathogens and autoimmune diseases.

Food contact materials

100 Groh *et al* (2017) reviewed the effect of food contact materials on gut health, including the gut microbiota. They recognised that a large number of potentially antimicrobial compounds that are used in food contact materials, such as packaging, or were added directly to foods have so far been insufficiently studied for any conclusions to be drawn. The polymer chitosan is known to be antimicrobial (the effect on Gram+ > Gram -) and has been shown to reduce the *Firmicutes* / *Bacteroidetes* ratio when consumed by pigs and humans. Other compounds reported to affect the composition of the microbiota in mammals, but without obvious antimicrobial properties, are diethylphthalate, methyl paraben, polysorbate-80 and carboxymethylcellulose. Many of these changes have been seen to be accompanied by gut inflammation.

Effects of the microbiota on xenobiotics.

101 The metabolism of xenobiotics from various sources by the gut microbiota is a two-edged sword, like that performed by host enzymes, in that it may lead to products that protect the host from chemical damage or convert relatively innocuous chemicals to active products. For example, sulfates and sulfur-containing amino acids in the diet may be reduced to hydrogen sulfide, which may lead to reduced functioning of colonic epithelial cells, inflammation and increased rates of colon cancer. Nitrate reduction results in the production of nitrite and thence nitroso compounds, which are known DNA alkylating agents. Conversely, metabolism of plant-derived flavonoids and glucosinolates by various genera of gut bacteria have been related to reduced levels of colorectal cancer (Hullar *et al*, 2014).

102 Li *et al* (2019) reviewed the methylation and demethylation of mercury by the gut microbiota of fish, terrestrial invertebrates and mammals. Papers from as early as 1975 showed that anaerobic incubation of inorganic mercury in closed off loops of

rat intestine and in the rumen of red deer could lead to methylmercury (MeHg) production. Incubation of HgCl₂ with a human faecal suspension under anaerobic conditions also produced MeHg and some of the bacteria capable of this process were identified as *Staphylococci*, *Streptococci* and *E. coli*. However, Zhou *et al* (2011, from abstract) found that human gut flora converted cinnabar (HgS) into less toxic mercuric polysulfides rather than MeHg, suggesting that the nature of the substrate can affect metallic species transformation and hence toxicity.

103 The gut biota of rats and marine fish are capable of methylmercury demethylation which confers protection against the neurotoxic effect of organic mercury in the diet. Guo *et al* (2018) tested the effect of methylmercury on the microbiota in slurries prepared from the faeces of two healthy human individuals (designated A and B) and found that there were marked inter-individual differences in demethylation. A balanced diet, a diet rich in carbohydrates and a diet rich in protein were tested on the ability of faecal slurry samples to demethylate methylmercury. The effect was enhanced by increased protein, but only in individual A. After 48 hours of methyl mercury treatment, *Proteobacteria* growth had reduced the proportion of the major phyla in individual A except in the high carbohydrate group, where there was marked growth of the *Actinobacteria*. The pattern in individual B was markedly different, with MeHg-carbohydrate having minimal impact on the microbiota profile but Hg and the other diets increasing the proportions of other minor phyla. On genetic profiling, the gut biota from neither individual expressed the *mer* operon, the best characterised mercury-resistance mechanism in bacteria, so the actual demethylation mechanism remained unresolved.

104 A number of reviews on the effects of the gut microbiota on the metabolism by, and thence influence in the pharmacological or toxicological effects of xenobiotics were identified in literature searches:

105 Saad *et al* (2012) listed a number of microbial metabolic actions that modify the effects of ingested chemicals such as enhancing the conversion of HAA into more potent mutagens, hydrolysing glucosides to release aglycones, potentiating host drug metabolism, activation of prodrugs and increasing or reducing toxicity depending on the drug in question.

106 Li and Jia (2013) listed the metabolic actions of microbiota-expressed enzymes on a range of drugs. Activities included reduction, hydrolysis, deacylation, deamination, proteolysis, ring opening and group scission. These actions led to xenobiotic activation or inactivation, increased absorption and increased activity, leading to either the desired therapeutic effect, reduction of this effect or unwanted toxicity.

107 Kim (2015) reviewed a range of drugs and phytochemicals on mechanisms of activation by microbial metabolism. Mechanisms covered are azo, nitro, sulfoxide, N-oxide, C=C, O-N and C-N reduction, deglycosylation, ring fission, desulfation, deamination, hydroxylation and dihydroxylation. Antibiotics potentiated some effects and antagonised others, although the mechanisms behind these differences were not discussed.

108 Currò (2018) reviewed the action of the gut microbiota on various pharmaceuticals, with examples. These included the activation of prodrugs, such as aminosalicilates by *Clostridium* and *Eubacterium* in the distal gut where the released drugs then act as anti-inflammatories against colitis, and anthranoid laxatives that are glycosides activated predominantly by *Bifidobacterium* species. Digoxin was reduced and deactivated predominantly by the species *Eggerthella lenta*. Bacterial β -glucuronidases have been implicated in the GI tract toxicity associated with the chemotherapeutic agent irinotecan and non-steroidal anti-inflammatory drugs.

109 Jourova *et al* (2016) reviewed a wide range of enzymatic transformations that are known to take place in the gut, presumably by the microbiota but only a few have been characterised to the genus or species level. Those identified in this paper were: paracetamol O-sulfation and C-S cleavage of paracetamol-3-cysteine by *Clostridium difficile*, reduction of digoxin by *Eggerthella lenta*, hydrolysis of the antiviral sorivudine by *Bacteroides* spp, nitro reduction of nitrazepam by *Clostridium leptum* leading to teratogenicity and increased activity of the anthelmintic levanosole by thiazole ring opening caused by *Bacteroides* and *Clostridium* spp.

110 The gut microbial metabolite *p*-cresol, derived from the protein amino acid tyrosine, appears to compete for the same O-sulfation pathway as paracetamol in the liver. The presence of a unique gut microbial community with different metabolic capacities may thus explain the difference in paracetamol metabolism and potential toxicity in different individuals (Clayton *et al*, 2009)

111 Velmurugan *et al* (2017) analysed the blood biochemistry of people who were occupationally exposed to organophosphates and dosed BALB/c mice with monocrotophos (MCP) to assess effects on glucose tolerance related to metabolism by the gut microbiota. In the workers, eighteen percent of the people directly exposed to OPs had diabetes compared with 6% of those indirectly exposed and half of those with the condition in each group had no family history of the disease. BALB/c mice were administered MCP orally in drinking water at 28 μ g/kg bw per day (10 times the theoretical maximum daily intake) for 180 days. The MCP treated mice showed increased blood glucose levels ($p < 0.0001$) beginning after 60 days' treatment without changes in body weight or plasma AChE activity. Lipid peroxidation, indicating oxidative stress, was also increased ($p < 0.01$). Faecal transplants between MCP-fed and control animals suggested that the gut microbiota were responsible for the observed glucose intolerance. The OP was found to induce faecal expression of enzymes for glucose and nucleotide metabolism, phosphate transport and vitamin biosynthesis. It was suggested that acetate produced by microbial metabolism of the OP was responsible for the gluconeogenesis, and a non-significant trend for higher acetate levels was found in the faeces of the diabetic OP workers.

112 Humblot *et al* (2007) investigated the possibility that bacterial β -glucuronidase could enhance the carcinogenesis of the food process genotoxic compound 2-amino-3-methylimidazo[4,5-*f*] quinoline (IQ) by reversing host phase 2 glucuronidation when the conjugate re-enters the gut lumen in bile. Gnotobiotic male F344 rats were gavage dosed with 1 ml of an overnight culture of *E. coli* TG1 that either expressed or was deficient in β -glucuronidase. The rats then received 90 mg/kg bw IQ or corn oil and were culled 4 hours later. Comet assays on colonocytes

and hepatocytes from the treated rats showed that the presence of β -glucuronidase led to a much longer tail length in the former cells than the latter, suggesting greater DNA damage. Thus, the authors suggested that the microbiota could play a role in the colonic carcinogenicity of food borne IQ, although the dose used is likely to greatly exceed that to which humans are exposed via the diet.

113 Beer *et al* (2019) examined the glycerol-dependant metabolism of heterocyclic aromatic amines (HAA) by the human faecal microbiota. HAAs are process contaminants of meat cooking, some of which are known experimental animal carcinogens and potential human carcinogens (IARC 2015). A range of HAAs were incubated with human faecal suspensions under strictly anaerobic conditions in the presence and absence of glycerol. Glycerol is known to be metabolised by various gut bacterial species, including *Lactobacillus*, to the reactive compound reuterin. Metabolites were analysed by RP-HPLC-TOF-MS/MS. A range of reuterin conjugation products were recovered. Reuterin conjugation blocks the exocyclic amino group of HAAs and is thought to reduce their ability to bind DNA.

Statins

114 Individuals are known to differ in their hypolipidaemic response to treatment with statins. Kaddurah-Daouk *et al* (2011) studied the potential genetic and non-genetic differences between good-and poor-responders to simvastatin in relation to the enteric metabolome. Plasma samples were analysed from participants in a clinical trial involving 944 Caucasian and African-American men and women with total cholesterol levels of 160 – 400 mg/dl (4.2 – 10.4 mmol/l), who were treated with 40 mg simvastatin/day for 6 weeks. There was a strong relationship between response to the statin and a higher level of secondary, bacterially derived, bile acids. The level of coprostanol, the reduced metabolite of cholesterol, produced in the gut also correlated positively with good response to the statin. The authors suggest that such knowledge could lead to developing microbiota-altering dietary interventions that could improve patients' response to statins.

115 The statin lovastatin is a prodrug that requires hydrolysis to its β -hydroxy metabolite to be activate as an HMG-CoA reductase-inhibitor. The observation that a cell-free preparation of rat or human faeces, known as fecalase, caused lovastatin degradation prompted Yoo *et al* (2014) to investigate the involvement of the gut microbiota in the activation of this drug. Both human and rat fecalase preparations were found to metabolise lovastatin, but when the rat preparation was made from the faeces of animals that had been treated with ampicillin or a mixture of cefadroxil, oxytetracycline and erythromycin, levels of activity were less than half that of the control. In live rats, the activity of three measured microbial enzymes – β -D-glucuronidase, α -L-rhamnosidase and β -D-glucosidase was reduced to almost zero after 3 days of antibiotic treatment. Metabolism of the statin by the gut microbiota contributed almost as much as that by the liver and the authors suggested therefore that patients who were co-administered an antibiotic would have a reduced level of the activated metabolite and hence reduced effectiveness of lovastatin. However, lovastatin activation is also known to take place in the serum (Tang and Kalow,

1995), which may result in continued activation of this statin in the absence of the microbiota

Probiotics

116 Unno *et al* (2015) fed six healthy female volunteers 2 servings daily of a fermented milk product containing *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Bifidobacterium longum*, *Lactobacillus casei* and *Streptococcus thermophilus* for three weeks. Faecal samples for analysis were collected from each volunteer at 3 time points; before ingestion of the product, at the end of the 3 week ingestion period and again 3 weeks later. By 16S rRNA gene sequencing of faecal samples, the *Bacteroidetes* increased in proportion, driven by increases in the *Bacteroidaceae* and *Pervotellaceae* families. At lower phylogenetic levels, the majority of the gut microbiota were little changed so that overall community stability was maintained.

117 Theilmann *et al* (2017) investigated the ability of a known human gut bacterium, *Lactobacillus acidophilus* to metabolise dietary plant glucosides. Their premise was that the aglycone moiety of the glucosides would then be secreted by the bacteria and serve as a substrate for further metabolism by other microbial species into bioactive products, which may be beneficial or toxic to the host depending upon the molecule. *L. acidophilus* grew on amygdalin, salicin (from willow bark), vanillin 4-O- β -glucoside (from vanilla), polydatin (from grapes), esculin (from dandelion coffee) and frexin (from kiwi fruit). The metabolism of amygdalin, esculin and salicin were compared. Lactate increased as the glucosides were metabolised and aglycones were produced in the external supernatant, with esculin and salicin being preferred substrates.

118 Wang *et al* (2018) observed that the bacterial strain *Bacillus cereus* BC7 protected mice against liver damage caused by the mycotoxin zearalenone. The bacterium was isolated from mouldy animal feed and was found to be able to utilise zearalenone as its sole carbon source, being able to degrade 90.4% of 2 mg/l zearalenone in 48 hours at 37°C. Degradation also took place in simulated gastric fluid. Female BALB/c mice were gavage dosed with saline, zearalenone (10 mg/kg bw), BC7 (6.9×10^7 cfu) or zearalenone-plus-BC7 once daily for 2 weeks after which the animals were euthanised and tissue, blood and faeces samples taken. Zearalenone markedly increased the titre of the *Bacteroidetes* at the expense of the *Firmicutes* and the co-administration of BC7 returned the balance almost to control levels although at genus level all of the treatments were markedly different from the control. Concomitant to this, BC7 ameliorated the liver damage caused by zearalenone in terms of histological appearance of the tissue, organ weight, and AST/ALT release. The authors suggested that BC7 could be used as a feed additive as a probiotic and a zearalenone removal agent.

Risk assessment and the microbiota

119 Considering the multitude of interactions between the gut microbiota and chemicals of various classes ingested with food, Licht and Bahl (2018) discussed how this knowledge may affect the risk assessment processes commonly used in

toxicology. In addition to the physiological differences between experimental animals and humans that lead to uncertainty in the extrapolation between species, the composition and metabolic capacity of the microbiota differ and overlap in unknown ways. To mitigate this, the authors suggested that animals used in toxicological studies could be modified by vendors in a number of ways:

- to each have as wide a range of microbiota to cover the widest range of activities;
- to have standardised microbiota or,
- each animal to have its microbiota analysed and correlation made between this and the findings of the experiment in that animal.
- Careful consideration should also be given to co-caging, randomisation and the effects of coprophagy and environmental contact on individual animals.

120 Velmurugan (2018) proposed a toxicological risk assessment protocol for the gut microbiota. The questions to be resolved were the effects of a chemical on the structure and function of the microbial community, the former of which could be addressed by whole genomic DNA isolation and 16S rRNA gene sequencing to assess dysbiosis and the latter by mass spectrometry techniques. The author outlined a workflow diagram of the steps he proposed. The use of germ-free mice that could be inoculated with human-like bacterial populations and the *in vitro* SHIME system were highlighted. The substrate used in the SHIME system could then be transplanted into a suitable host animal to assess the effect of the changes observed *in vitro* in an intact animal. Single bacteria or whole communities could also be assessed by “gut on a chip” microfluidic technology, as described by Kim *et al* (2016).

121 Kim *et al* (2016) described a “gut on a chip” system where cultured intestinal epithelial cells and microbiota were seeded on a support inside a flow-cell device. The walls of the flow cell were deformed by rhythmic alterations in external pressure to simulate peristalsis, so that the system more closely resembled the normal structure and function of the gut. The cells and bacteria inside this system developed in a more naturalistic way than seen in a static system. Chemical and biological stresses could then be applied and changes to the system could be assessed.

Derivation of microbiological health-based guidance values

122 The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidance document GL36(R2) (2019) outlines recommendations for deriving a microbiological acceptable daily intake (ADI) for dietary residues of a veterinary medicine with suspected antimicrobial properties. The procedures for deriving ADIs, based on either disruption of the intestinal colonisation barrier or on overgrowth of drug resistant species, using *in vivo* or *in vitro* methods, are outlined. This guidance updates guidance GL36(R) (2004, implemented 2013).

123 In their 2018 report, the Joint Food and Agriculture Organization / World Health Organization Meeting on Pesticide Residues (JMPPR) stated that “The use of pesticides, particularly fungicides, in agriculture to control plant pathogens in crops

could result in residues in food, which, on ingestion, may interact with the microbiome in the human gastrointestinal tract...” JMPR recommended that studies of the effects of pesticides on the intestinal microbiota should be considered, and that this should follow the same step-wise decision-tree approach used by the Joint Food and Agriculture Organization / World Health Organization Expert Committee on Food Additives (JECFA) when establishing a microbiological ADI and ARfD for residues of veterinary drugs.

124 The 85th JECFA meeting report recommended that “...studies be conducted according to internationally recognized standards using at least 10 strains of the relevant genera of intestinal bacteria sourced from faecal samples of healthy donors taking into consideration recent scientific knowledge from molecular and metagenomic studies on intestinal microbial community composition; and that *in vitro* or *in vivo* studies be conducted using a range of concentrations of the antimicrobial agent, from residue levels to therapeutic levels, and that these studies address the effects.” (JECFA 2018).

125 Points to be considered before a microbiological HBGV for a particular xenobiotic (as applied by JECFA to residues of veterinary drugs) would be considered necessary are:

- Are residues of the drug and/or its metabolites microbiologically active against representatives of the human intestinal flora?
- Do residues enter the human colon?
- Do the residues entering the human colon remain microbiologically active?

126 JECFA produced a guidance document on the derivation of a microbiological acute reference dose (ARfD) (JECFA, 2016). Disruption of the colonisation barrier is the endpoint relevant to acute exposure and therefore would normally be the basis of a microbiological ARfD.

127 The calculations for microbiological ADIs and ARfDs are similar to one another in format. For the derivation relating to the use of *in vitro* models using defined bacterial strains of bacteria, the formula derived by JECFA is as follows:

$$\text{HBGV} = \frac{\text{POD (MIC}_{\text{calc}} \text{ or NOAEC)} \times \text{correction factors} \times \text{colon volume}}{\text{Fraction of oral dose available to microbiota} \times \text{body weight}}$$

Where:

HBCV = health-based guidance value (ADI or ARfD)

POD = Point of Departure = Minimum Inhibitory Concentration or No-Observed-Adverse-Effect-Concentration.

MIC_{calc} = calculated minimum inhibitory concentration. MIC_{calc} represents the lower 90% confidence limit for the mean MIC₅₀ (the minimum inhibitory concentration for 50% of strains) for the 10 most relevant and sensitive

human colonic bacterial genera. An intrinsically resistant bacterial genus should not be included.

Correction values (where appropriate) take into account considerations not used for the microbiological ADI that may be appropriate to the microbiological ARfD. For example, a factor of 3 to allow for temporal dilution during gastrointestinal transit and for dilution by consumption of additional meals. Others may take into account the inoculum effect on MIC determinations, pH effects on the MIC, and possibly other physico-chemical-specific factors of the growth conditions used in testing.

The fraction of an oral dose available for colonic microorganisms should be based on *in vivo* measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine.

The value assumed for the volume of the colon has recently been increased from 220 ml to 500 ml, based on a review of the scientific literature.

Body weight = 60 kg.

128 JECFA (2018) stated that "... data from *in vitro* studies (continuous culture flow chemostats) and *in vivo* models (human volunteers, animal models and human microbiota-associated animals) are evaluated by the Committee for both microbiological end-points. However, data from these studies can be problematic in determining a microbiological ADI and/or ARfD. This is due to the small sample size in the animal studies; insufficient data and low power of studies in human volunteers (because of small numbers of subjects); concentrations of antimicrobial agent generally not being adequate to determine a chronic or acute dose with no effect; and the lack of validation of the *in vitro* and *in vivo* test models....and... Therefore, the Committee recommends that *in vitro* or *in vivo* studies be conducted using a range of concentrations of the antimicrobial agent, from residue levels to therapeutic levels. Such studies should address the predominant bacterial strains that inhabit the gastrointestinal tract when determining if levels of antimicrobial residues in animal-derived food after consumer ingestion can increase the population of antimicrobial-resistant intestinal bacteria in the gastrointestinal tract."

Summary and conclusions

129 The composition of the microbial community of the gastrointestinal tract is complex, consisting of a relatively small number of major phyla, within which the taxonomic groups account for an enormous range of species. The number and range of species present depends upon the local conditions and thus position in the gut, the major repository being the large intestine, particularly the caecum.

130 Some information on the non-bacterial components of the gut microbiota (fungi, viruses, protozoa and *archaea*) is available in the scientific literature but their interactions and possible contributions to the structure and function(s) of the flora as a whole are presently much less well characterised than those of the bacteria.

131 The majority of investigations into the effects of xenobiotics on the gut microbiota have utilised animal models, the data from which have been used to make statements about possible effects on the flora of humans. Such experiments have been performed as they have because of the favourable characteristics of the model (ease of handling and dosing, the ability to use large dosage groups, analysis of gut compartments) and because it would not be possible or ethical to perform similar studies on humans.

132 It is known from animal studies that the mobile luminal population of bacteria in the gut differs from the more fixed mucosal population so that changes in the faecal microbiota may not reflect changes in the whole community. Therefore, *ex vivo* model systems such as SHIME address changes only in the more tractable part of the bacterial community and still give an incomplete estimate of changes in the community as a whole.

133 Although animal work has shown that many different types of xenobiotic appear to affect the balance of the gut microbiota, there is little unequivocal evidence as to whether these changes are pathological, or at least have any physiological significance to the host or are adaptive in nature. In addition, it is largely unclear on how to extrapolate effects observed at the often high doses used in animal studies to typical human exposure levels.

134 Human studies have shown that although the balance of the measurable microbiota can be altered by the presence of ingested xenobiotics, even many of the changes brought about by oral antibiotics have been observed to be smaller than differences between apparently healthy individuals

135 Despite sometimes large-scale variability in the composition between healthy individuals' gut microbiota as well as in response to xenobiotics or in the presence of conditions such as inflammatory bowel disease, there appears to be redundancy of functionality due to different taxa of bacteria expressing different gene products with comparable activities. Thus, structural dysbiosis, depending upon the taxa affected, may not always lead to a large change in overall function causing detriment to the host.

136 The gut microbiota also appear to participate in the activation and deactivation of ingested substances, including effecting or affecting the action of some pharmaceuticals, and thus may lead to toxicity, or to intended, reduced or prolonged pharmacological action.

137 The presence of some bacterial taxa has been associated with metabolic defects in the host and others with good health and barrier function of the epithelium, but the mechanisms involved have yet to be fully elucidated. The change in the balance of gut bacteria and the development of diseases such as type 2 diabetes,

obesity and neurological deficits is so far correlative, without definitive evidence of causation. Nevertheless, this is an area of active research with potential major implications for understanding of the pathophysiology of a number of major human diseases.

138 Given the current interest in personalised medicine, it is possible that attempts will be made to utilise an individual's gut microbiota to tailor treatments for gastrointestinal or systemic pathologies in which they or their metabolic capacity are purported to be involved. Achieving this would also allow a precise, personal risk assessment of the toxicity of an encountered xenobiotic. This, however, would require knowledge of the organisms and the causal links involved, which in most cases is currently unavailable.

139 The Committee recognises that the human gut microbiome is currently an area of major research focus and will keep the subject under review, particularly with respect to possible implications for chemical risk assessment.

Secretariat

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Appendix 1

Search terms in PubMed

The search terms used for this paper were largely of the format:

Microbiome OR microbiota AND “X” and human AND toxicity AND gut, where “X” was:

Heavy metal	Dysbiosis
Insecticide	Sweeteners
Herbicide,	Bisphenol A
Xenobiotic,	Chlorpyrifos
Pyrethroid	Gold
Organophosphate	Tin
DDT	Mercury
DEET	Antimony
Glyphosate	Nickel
Food contact materials,	Silver
Polyamines	Titanium
Drug metabolism	PAHs
Food additive	Emulsifiers
Antibiotics	Probiotic
Alcohol	Prebiotic

Function

Composition

Cocciostat Some references were found in the reference lists of papers

Fungicide acquired in the PubMed searches.

Aldrin

Dieldrin

Flame retardant

Metabolite

Mycobiome OR fungi AND "X" AND human AND toxicity AND gut
Virome OR viruses AND "X" AND human AND toxicity AND gut

Appendix 2

Abbreviations

5HT	5-hydroxytryptamine, serotonin
ABC	ATP-Binding-Cassette trans-membrane transport protein
ACh	Acetylcholine
AChE	Acetylcholinesterase
ADI	Acceptable daily intake
AFB1	Aflatoxin B1
AgNP	Silver nanoparticles
AgOAC	Silver acetate
AHR	Aromatic hydrocarbon receptor
ALT	Alanine aminotransferase
AMR	Antimicrobial resistance
ARfD	Acute reference dose
ARG	Antimicrobial resistance gene
As	Arsenic
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AuNC	Gold nanoclusters
BC	<i>Bacillus cereus</i>
BDE	Brominated diphenylether
BPA	Bisphenol A
BS	<i>Bacillus subtilis</i>
CCAAT	A DNA transcription initiation site
Cd	Cadmium
C/ERP α	CCAAT/enhancer-binding protein alpha
cfu	Colony-forming units
CNS	Central nervous system
CPF	Chlorpyrifos
Cr	Chromium
CYP	Cytochrome P450
DDT	Dichlorodiphenyltrichloroethane
DMA	Dimethylarsinic acid
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DOM-1	Deepoxydeoxynivalenol
DON	Deoxynivalenol
DONGlc	Deoxynivalenol-3-glucoside
DSS	Dextran sodium sulfate
EE	Ethinyl (o)estradiol
F1	First generation offspring
FAO	United Nations Food and Agriculture Organization
FBG	Ferrous bisglycinate
Fe	Iron
FeEDTA	Ferrous ethylenediaminetetraacetic acid
FOXP3	Forkhead immune-regulatory protein P3
FS	Ferrous sulfate
FXR	Farnesyl-X receptor
GC	Gas chromatography

GERD	Gastrointedinal-esophageal reflux disease
GI	Gastrointestinal
GPR	Orphan G-protein-coupled receptor
HAA	Heterocyclic aromatic amines
HBCDD	Hexabromocyclododecane
HDL	High-density lipoproteins
Hg	Mercury
HgCl ₂	Mercuric chloride
HgS	Mercuric sulfide, cinnabar
HMG-CoA	Hydroxymethylglutarate coenzyme A
HPA	Hypothalamic-pituitary-adrenal
HRS	High-resistant starch
IARC	International Agency for Research on Cancer
IBD	Inflammatory bowel disease
Ig A	Immunoglobulin A
IL	Interleukin
IQ	2-amino-3-methylimidazo[4,5-f]quinoline
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC	Liquid chromatography
LDL	Low-density lipoproteins
LPS	Lipopolysaccharide
MCP	Monocrotophos
MeHg	Methyl mercury
MIC	Minimum inhibitory concentration
μM	Micromolar
μm	Micrometre
MMA	Monomethylarsonic acid
Mn	Manganese
MP	Microplastic
mRNA	Messenger RNA
MS	Mass spectrometry
nM	Nanomolar
NO	Nitric oxide
NOAEL	No-observed-adverse-effect-level
NOD	Interbacterial flavonoid signalling receptor
NP	Nanoparticle
NSAID	Non-steroidal anti-inflammatory drug
OP	Organophosphate
p,p-DDE	Dichlorodiphenylethylene
PAH	Polycyclic aromatic hydrocarbon
Pb	Lead
PBDE	Polybrominated diphenylether
PBS	Phosphate-buffered saline
PCB	Polychlorinated bisphenyl
PCR	Polymerase chain reaction
PD	Parkinson's disease
PFOS	Perfluorooctane sulfonic acid
PhIP	2-amino-1-methyl-6-phenylimidazole[4,5-b]pyridine
PiP3	Phosphatidylinositol-3,4,5-trisphosphate

PM	Propamocarb
PM2.5, 10	Airborne particles of 2.5, 10 μm diameter
PND	Post-natal day
PTEN	Phosphatase and tensin homolog
PXR	Pregnane-X receptor
Q-PCR	Quantitative polymerase chain reaction (aka RT-PCR)
Reg III	An antimicrobial lectin of the Regulatory protein, LysR-family
RP-HPLC	Reversed-phase high performance liquid chromatography
rRNA	Ribosomal riboneucleic acid
RT-PCR	Real-time polymerase chain reaction
S	Svedberg unit of centrifugal sedimentation time (10^{-13} seconds)
SCFA	Short-chain fatty acid
SPF	Specific-antigen-free
spp	Species (plural)
Srebp1	Sterol regulatory element-binding protein
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TCDF	2,3,7,8-tetrachlorodibenzofuran
TiO ₂	Titanium dioxide
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TOF	Time-of-flight
UDP	Uridine diphosphate
UPLC	Ultra Performance Liquid Chromatography
VLDL	Very-low-density lipoproteins
WHO	World Health Organization

Table 1. Recently reported experiments on the effects of metals on the gut microbiota of mice and rats

Animal	Metal	Effect	Reference
C57BL/6 mice	Iron	Decrease in proinflammatory <i>Desulfovibrio</i> , increase in anti-inflammatory <i>Bifidobacterium</i> .	Werner et al (2010)
C57BL/6 mice		Iron was pro-or anti-inflammatory depending on formulation, various genera changed	Constante et al (2017)
C57BL/6 mice		Reduced <i>Bacteroidetes</i> and <i>Firmicutes</i> , increased <i>Proteobacteria</i> and <i>Actinobacteria</i>	Mahalal et al (2018)
ICR mice	Iron plus arsenic	Increase in <i>Firmicutes</i> , decrease in <i>Bacteroidetes</i> and <i>Actinobacteria</i> .	Guo et al (2014)
C57BL/6 mice	Arsenic (10 ppm or 250 ppm As over 5 weeks)	Increase in <i>Bacteroidia</i> , decrease in <i>Clostridia</i> classes with minor classes growing or receding	Dheer et al (2014)
C57BL/6 mice	(10 ppm As)	In females, up-regulated Hg- for Zn-resistance genes and trans-membrane transporters. In males, hexose phosphate uptake down-regulated and denitrification up-regulated	Chi et al (2016)
C57BL/6 mice	Arsenic with zinc deficiency (50 < 500 ppm As)	Reduced levels of zinc sensitised the microbiota to the effects of arsenic. Zinc deficiency increased arsenic- induced DNA damage and oxidative stress	Gaulke et al (2018)
C57BL/6 mice	Manganese (20 mg Mn/kg bodyweight (bw)/day)	Sex-specific disruption of the normal structure and function of the microbiota, changes to quorum-sensing affected population density, motility and virulence; and enriched some species; induction of oxidative stress, changed iron homeostasis	Chi et al (2017)
Balb/c mice	Cadmium.(23 to 50 mg Cd/kg bodyweight)	Reduced titre of culturable anaerobic and aerobic bacteria in the small intestine, large intestine and rectum. Gram negative bacteria more resistant to cadmium than Gram positive	Fazeli et al (2011)
Balb/c mice	(20 and 50 mg Cd/kg bodyweight)	Reduced <i>Firmicutes</i> / <i>Bacteroidetes</i> ratio, and population of <i>Lactobacilli</i> and <i>Bifidobacteria</i> . Intestinal mucus layer thinning, increased colon TNF α , reduced production of SCFA.	Liu et al (2014)
C57BL/6 mice	(100 nM Cd)	Fat mass increased and microbial diversity decreased. Plasma triglycerides, total cholesterol, free FA, leptin HDL and liver triglycerides increased. <i>Bacteroidetes</i> increased and <i>Firmicutes</i> decreased.	Ba et al (2017)
Balb/c mice		No effect on bacterial diversity by 16S rRNA analysis in the caecum and faeces of female Balb/c mice. The relative proportions of different families and genera markedly affected	Breton et al (2013a)

Germ-free and SPF C57BL/6 mice	Cadmium plus lead	The presence of the gut biota may have led to reduced uptake of metals in the germ-free mice.	Breton et al (2013b)
C57BL/6 mice	Lead (32 ppm Pb in drinking water)	<i>Bacteroidetes</i> decreased and <i>Firmicutes</i> increased. Fewer culturable aerobes and more culturable anaerobes in the faeces	Wu et al (2016)
C57BL/6 mice	(2mg Pb/kg bodyweight/day)	Population diversity was reduced, Levels of vitamin E and bile acids were reduced. urea decreased and copper -containing nitrite reductase was induced. Gluconeogenesis decreased. Oxidative stress and phosphate ABC transporter genes increased	Gao et al (2017)
ICR mice		Caecal <i>Firmicutes</i> were reduced by <0.1 mg/l lead whereas <i>Bacteroidetes</i> reduced only at 0.1 mg/l. <i>Proteobacteria</i> and <i>Actinobacteria</i> unaffected	Xia et al (2018)
Sprague Dawley rats	Copper	Marginal (1.5 ppm) and supplemented (20 ppm) copper in the diet of male weanling Sprague Dawley rats increased the gut <i>Firmicutes/Bacteroidetes</i> ratio but different families and genera within the Firmicutes phylum were responsible for the change in ratio of the phyla in each treatment	Song et al (2017)
C57BL/6 mice	Titanium (food grade TiO ₂)	Minor changes: <i>Parabacterioides</i> , <i>Lactobacilli</i> and <i>Allobaculum</i>) increased, <i>Aldercreutzia</i> and unclassified <i>Clostridiaceae</i>) decreased. Acetate production decreased, trimethylamine increased. Biofilm production increased	Pinget et al (2019)
Mice	Silver (NP) (46, 460 and 4600 ppb AgNP)	Dose dependent increased ileal <i>Firmicutes/Bacteroidetes</i> ratio, Balance of genera changed Older NP less effective possibly due to sulfidation	Van den Brule et al (2016)
Sprague Dawley rats	(9, 18 and 36 mg/kg bodyweight of 10 nm AgNP for 3 weeks)	<i>Bacteroidetes</i> and <i>Firmicutes</i> reduced, males more sensitive than females. Decreased activity of genes for T-cell activity, mucin and microbial recognition in the gut.	Williams et al (2016)
C57BL/6 mice	(10 mg/kg bodyweight of 110 nm particles for 24 days)	No significant changes	Wilding et al (2016)
SPF Balb/c mice	Gold (NP)	Increase in <i>Proteobacteria</i> . (<i>Roseburia</i> were depleted and <i>Staphylococcus</i> , <i>Ureoplasma</i> and <i>Methylobacterium</i> were more abundant). Decrease in butyrate production and an increase in gut inflammation.	Wang et al (2019)

Table 2 Reported experiments on the effect of pesticides on the gut microbiota of mice and rats.

Animal	Pesticide	Effect	Reference
C57BL/6 mice	p, p'-DDE and β -HCH	Reduced <i>Actinobacteria</i> and the <i>Candidatus Saccharibacteria</i> no effect on <i>Bacteroidetes</i> , <i>Firmicutes</i> , <i>Verrucomicrobia</i> or <i>Proteobacteria</i> .	Liu <i>et al</i> (2017)
Male ICR mice	Endosulfan (0.5, and 3.5 mg/kg for 2 weeks)	Serum hippurate levels fell dimethylalanine and trimethylamine N-oxide increased.	Zhang <i>et al</i> (2017)
Wistar rats	Permethrin (75:25 trans:cis)	Various <i>Bacteroidetes</i> <i>Lactobacillus</i> spp temporarily increased <i>Bacteroidetes</i> spp were significantly reduced	Nasuti <i>et al</i> (2016)
Mice	Chlorpyrifos (5 mg/kg bodyweight/ day for 4 weeks)	Chlorpyrifos treatment of mice on a normal diet had symptoms similar to those on the high fat diet.	Liang <i>et al</i> (2019)
Wistar rats	(0.3 or 3.0 mg /kg bodyweight/day for 9 weeks)	Low dose chlorpyrifos (0.3 mg/kg bw) affected the balance of bacterial genera more than did a higher dose (3 mg/kg bw) Gut AChE activity reduced.	Fang <i>et al</i> (2018)
Wistar rats	(1 and 3.5 mg/kg bodyweight /day)	Reduced <i>Firmicutes</i> and other SCFA-producing bacteria partially reversed by inulin	Reygner, <i>et al</i> (2016)
SPF C57BL/6 mice	Diazinon (4 mg/l in drinking water for 13 weeks)	Sex-specific changes in the relative titres of gut microbiota the genus level. some species markedly decreased in males, but the majority increased, and the majority decreased in females., serotonin metabolic pathway gene expression perturbed.	Gao <i>et al</i> (2017)
SPF C57BL/6 mice	Malathion	Genes for quorum sensing flagellar proteins pathogenicity and virulence. upregulated	Gao <i>et al</i> (2018)
SPF C57BL/6 mice	Aldicarb	Genes for virulence, profiles of diglycerides, triglycerides and phosphatidylcholines in the liver, faeces and brain disturbed. increased expression of oxidative stress-related genes in the gut, protein degradation and DNA damage.	Gao <i>et al</i> (2019)
Male Sp rague Dawley rats	Glyphosate (Up to 25 mg/kg bodyweight)	Little effect on the bacterial populations on the ileum, caecum and colon probably due to the presence of already-adequate amounts of aromatic amino acids.	Nielsen <i>et al</i> (2018)
Sprague Dawley rats	(0.1 ppb, 400 ppm and 5000 ppm in drinking water)	<i>Bacteroidetes</i> family S24-7 increased. <i>Lactobacilliaceae</i> decreased in 8 out of 9 treated animals. In vitro, <i>Bifidobacteria</i> , <i>Clostridia</i> and <i>Enterococci</i> were sensitive to glyphosate at 400 ppm, <i>Lactobacilli</i> above 5000 ppm and coliforms not sensitive	Lozano <i>et al</i> (2018)
Male Swiss mice	(250 or 500 mg/kg bodyweight/ day)	<i>Firmicutes</i> , <i>Corynebacterium</i> , <i>Bacteroidetes</i> spp and <i>Lactobacillus</i> spp depleted.	Aitbali <i>et al</i> (2018)

Sprague Dawley rats	Glyphosate (1.75 mg/kg bodyweight/ day)	Significant changes in the balance of the microbiota in the glyphosate- and Roundup™-treated dams and pups compared with a water control	Mao <i>et al</i> (2018)
ICR and C57Bl/6 mice	Imazalil (100 mg/kg bodyweight for 1, 7 and 14 days)	Fall in the relative abundance of the <i>Bacteroidetes</i> at all imazilil doses and in the <i>Firmicutes</i> and <i>Actinobacteria</i> at the highest dose and later time points; increases on <i>Firmicutes</i> , α - <i>Proteobacteria</i> and γ - <i>Proteobacteria</i>	Jun <i>et al</i> (2016 and 2018)
C57Bl/6 mice	Fluconazole (0.5 mg/ml in drinking water)	Decrease in <i>Candida spp</i> , increase in <i>Aspergillus</i> , <i>Wallemia</i> and <i>Epicoccum</i> exacerbation of DSS-induced colitis and house-dust-mite-extract-induced allergic airway disease	Wheeler <i>et al</i> (2016)
ICR mice	Propamocarb	<i>Bacteroidetes</i> α - <i>Proteobacteria</i> and γ - <i>Proteobacteria</i> reduced <i>Firmicutes</i> increased at 3 mg/l and then fell. In the caecum the <i>Actinobacteria</i> and β - <i>Proteobacteria</i> fell but little change in the other phyla at 300 mg/l	Wu <i>et al</i> (2018a)
ICR mice	Propamocarb (1, 3 and 20 mg/l in drinking wayer)	Faecal expression of genes involved with bile acid synthesis and transport increased. trimethylamine levels were increased	Wu <i>et al</i> (2018b)
Female Sprague Dawley rats	Epoxiconazole 4 and 100 mg/kg bodyweight for 90 days)	Increasing <i>Bacteroidetes</i> and the <i>Proteobacteria</i> decrease in <i>Firmicutes</i> (Xu <i>et al</i> (2014)
SPF C57BL/6 mice	2,4-dichlorophenoxyacetic acid (2,4-D) (15 mg/kg bodyweight)	Increased <i>Bacteroidetes</i> , <i>Chlorobi</i> , <i>Chloroflexi</i> , <i>Spirochetes</i> and <i>Thermotogae</i> no change in <i>Acidobacteria</i> ,	Tu <i>et al</i> (2019)

Table 3. Reported experiments on the effects of antibiotics on the gut microbiota of mice and rats.

Animal	Antibiotic	Effect	Reference
Male C57BL/6 mice	Ampicillin (30 mg/kg bw) and tetracycline (2 and 50 mg/kg bw)	Gut antibiotic resistance increased with treatment but for ampicillin, but the effect was smaller for iv than for oral dosing. Tetracycline, however, is excreted by both the kidney and the GI tract, so the microbiota were exposed by both routes of treatment.	Zhang <i>et al</i> (2013)
Pregnant C57 Bl/6 mice	Penicillin V (31 mg/kg bw / day)	<i>Actinobacteria</i> decreased, <i>Bacteroidetes</i> and <i>Firmicutes</i> both increased	Leclercq <i>et al</i> (2016)
Male and female Wistar rats	4-EOTC, a major oxytetracycline metabolite (5 or 50 mg/kg bw)	Fall in <i>Bacteroidetes</i> . <i>Actinomycetes</i> (<i>Bifidobacteria</i>) increased. The families <i>Lactobacilliaceae</i> (<i>Helicobacteriaceae</i>) depleted. Tetracycline resistance increased	Han <i>et al</i> (2016)
Male Sprague Dawley rats	Ampicillin (alone at 90 mg/kg bw), or cocktail of ampicillin, neomycin, gentamicin, metronidazole (all at 1.75 mg/kg bw) and vancomycin (0.875 mg/kg bw), then mixed atrazine, simazine, ametryn, terbutylazine and metribuzin (all at 2 or 20 mg/kg bw).	Increased bioavailability of all the triazines without affecting microbial or hepatic triazine metabolism.	Zhan <i>et al</i> (2018)
Weanling C57BL6 mice	Penicillin, vancomycin, penicillin-plus-vancomycin or chlortetracycline for 7 weeks, all at 1 mg/kg bw in drinking water	<i>Lachnospiraceae</i> family increased, <i>F/B</i> ratio increased. Butyryl CoA transferase copy number decreased	Cho <i>et al</i> (2012)

		at 3 weeks but increased, along with SCFAs by 6 weeks,	
Female BALB/c mice	Ciprofloxacin (cip) and metronidazole or vancomycin in drinking water for 3 weeks	<i>Bacteroidetes</i> and <i>Actinobacteria</i> depleted, <i>Proteobacteria</i> increased. <i>F/B</i> increased. <i>Streptococcaceae</i> increased with cip, <i>Lactobacillaceae</i> and <i>Enterobacteraceae</i> increased with vancomycin	Nagano <i>et al</i> (2019)

Table 4 Reported experiments on the effects of various xenobiotics on the gut microbiota of mice and rats

Animal	Xenobiotic	Effect	Reference
C57BL/6 mice	Morphine, slow release pellets (serum concentration ~ 1 μ M)	Reduced <i>Bacteroidetes</i> . In the <i>Firmicutes</i> , <i>Enterococcaceae</i> , <i>Staphylococcaceae</i> , <i>Bacillaceae</i> , <i>Streptococcaceae</i> and <i>Erysipelotrichaceae</i> increased. Gut barrier negatively affected and bacteria (mainly Gram positive) translocated through the epithelium	Banerjee <i>et al</i> (2016)
C57BL6 , TLR5 ^{-/-} and IL10 ^{-/-} mice	Carboxymethylcellulose (CMC) or polysorbate-80 (P80) (at 1% w/v or v/v in drinking water for 132weeks)	Mucus layer degradation and contact of bacteria with the gut epithelium. Reduced <i>Bacteroidetes</i> , increased <i>verrucomicrobia</i> and <i>Proteobacteria</i> . Low-grade inflammation and metabolic disease in WT mice and increased incidence/severity of overt colitis in interleukin-10 (IL-10) ^{-/-} mice,	Chassaing <i>et al</i> (2015, 2016)
Male C57BL6 mice	Carboxymethylcellulose (CMC) or polysorbate-80 (P80) (at 15 w/v or v/v in drinking water for 13 weeks)	<i>Firmicutes</i> decreased, <i>Bacteroidetes</i> increased, making conditions more pro-inflammatory.	Viennois <i>et al</i> (2017)
Female C57BL/6 N mice	Soy or coconut oil (25%w/w oil in diet)	Coconut-oil-fed mice had higher blood cholesterol level after 8 weeks. and a greater relative abundance of <i>Allobaculum</i> and <i>Anaerofustis</i> and depletion of <i>Akkermansia</i> compared with soy oil.	Patrone <i>et al</i> (2018)

Male and female CD-1 mice	Acesulfame-K (37.5 mg/kg bw/day by gavage for 4 weeks)	Various genera affected. carbohydrate absorption and metabolism genes decreased in females and increased in males. In male mice inflammation and virulence genes upregulated. Organic acid and bile acids were affected differently in male and female mice	Bian <i>et al</i> (2017)
Mouse model of Cohn's disease	Splenda (1% sucralose, 99% maltodextrin w/w) (1.08, 3.5 or 35 mg/kml in drinking water for 6 weeks)	Over growth of 5 classes within the phylum <i>Proteobacteria</i> . No significant change to <i>Firmicutes</i> or <i>Bacteroidetes</i> .	Rodriguez-Palacios <i>et al</i> (2018)
Male C57BL6 mice	Sucralose (0.1 mg/ml in drinking water for 6 months, equivalent to 5 mg/kg bw/ day in humans)	Genes for LPS synthesis and flagellar components and fimbriae were up-regulated	Biamnet <i>et al</i> (2017)
Male C57BL/6 mice	Xylitol (40 or 200 mg/kg bw/day for 18 weeks in conjunction with low- or high fat diet)	No significant changes	Uebanso <i>et al</i> (2017)
Male Sprague Dawley rats	Ethanol (2 g/kg bw/ day, escalating over 2 weeks to 8g/kg bw/day for a further 8 weeks)	Significant changes (p<0.05) to diversity, richness and evenness of the colonic population Addition of oats or the probiotic <i>Lactobacillus</i> , maintained parameters at control levels	Mutlu <i>et al</i> (2009)
Male Sprague Dawley rats	Ethanol in liquid diet at 5%, 5.14%, 5.29% and 5.43% w/v for 1–2, 3–4, 5–6 and 7–8 weeks, respectively	The distribution of amino acids, fatty acids and steroids changed: branched-chain amino acids increased and SCFAs, (acetate), increased in the stomach and colon. Other metabolites reduced in all compartments	Xia <i>et al</i> (2013)
Male C57BL/6 mice	(Liquid diet rising from 0 to 5% (w/v) ethanol over 2 weeks, maintained for further 6 weeks)	Reduced <i>Firmicutes</i> and <i>Bacteroidetes</i> , over growth of the <i>Actinobacteria</i> and <i>Proteobacteria</i>	Bull-Otterson <i>et al</i> (2013)
Female C57BL/6 mice	(5%(w/v) ethanol in liquid diet for 10 days)	No changes in bacterial diversity but an increase in the <i>Actinobacteria</i> and a decrease in <i>Verrucomicrobia</i>	Lowe <i>et al</i> (2017) and Lowe <i>et al</i> (2018)
Male Sprague Dawley rats	Aflatoxin B1 (AFB1) (25 µg/kg bw 5 days a week for 4 weeks)	Slight reduction in <i>Bacteroidetes</i> minor increases in <i>Firmicutes</i> and <i>Proteobacteria</i> but marked change at the genus level.	Liew <i>et al</i> (2019)

Male F344 rats	(5, 25 or 75 µg/kg bw/ day by gavage 5 days a week for 4 weeks)	Reduced genetic diversity No major phylum level shifts but <i>Firmicutes</i> species were unchanged or increased, but lactic acid bacteria, reduced, Bacteroidetes unchanged or slightly reduced	Wang <i>et al</i> (2015)
Male C57BL/6 mice	BaP (50 mg/kg bw by gavage for up to 27 days)	Faecal <i>Bacteroides</i> increased and <i>Verrucomicrobia</i> decreased. <i>Lactobacillus</i> and <i>Akkermansia</i> , decreased in t faeces. Different mucosal taxa increased and decreased, depending upon location.	Rivière <i>et al</i> (2016)
Male C57BL/6 mice	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin (TCDD). (0.01, 0.03, 0.1, 0.3, 1, 3, 10, or 30µg/kg TCDD by gavage every 4 days for a total of 28 days)	Increased relative abundance of species in the genera <i>Lactobacillus</i> , <i>Clostridium</i> , <i>Streptococcus</i> and <i>Listeria</i> .	Fader <i>et al</i> (2017)
C57BL/6 mice (<i>Ahr</i> ^{-/-} and <i>Ahr</i> ^{+/+})	2,3,7,8-tetrachloribenzofuran (TCDF) (24 µg/kg bw in diet for 5 days)	Decreased the <i>Firmicutes</i> / <i>Bacteroidetes</i> ratio without affecting the <i>Actinobacteria</i> .	Zhang <i>et al</i> (2015)
Male <i>Ldtr</i> ^{-/-} mice	Polychlorinated biphenyl126 (PCB 126) (1 µmol/kg via oral gavage at weeks 2 and 4 of a 12 week study.)	Reduced bacterial diversity with reductions in <i>Bifidobacterium</i> , <i>Lactobacillus</i> and <i>Ruminococcus</i> but an increase in <i>Akkermansia</i> . Inflammatory cytokines were increased,	(Petriello <i>et al</i> , 2018).
Female CD-1 mice	Perfluorooctane sulfonic acid (PFOS) (0.3 or 3 µg/g/day for 7 weeks.)	No effect on bacterial diversity. Some taxa increased and others decreased. Low-PFOS dose induced increase in the genus <i>Turicibacter</i> , The genus <i>Allobaculum</i> , a SCFA-producing genus also increased	Lai <i>et al</i> (2018)
Conventional and germ-free C57BL/6 mice	Polybrominated diphenyl ethers (PBDE), (BDE-47 (100 mmol/kg), or BDE-99 (100 mmol/kg) via oral gavage once daily for 4 consecutive days	Metabolism of PBDEs by mouse liver was modified by the presence or absence of gut microbiota	Li <i>et al</i> (2017)
Pregnant female ICR mice and their offspring	Triphenylphosphate (TPHP)(10, 100, and1000 µg/kg bw)	Increased bacterial classes <i>Erysilelotrichia</i> and <i>Bacilli</i> and decreased <i>Clostridia</i> . Genera <i>Allobaculum</i> , <i>Tunicibacter</i> and <i>Lactobacillus</i> increased.	Wang <i>et al</i> (2019)

Male C57BL/6 mice	Mono-2-ethylhexylphthalate ester (MEHP) (0.05 mg/kg bw)	Increase in <i>Firmicutes</i> , reduced <i>Verrucmicrobia</i> and increase in the <i>Firmicutes/Bacteroidetes</i> ratio. Reduced <i>Akkermansial</i> and <i>Alloprevotella</i> genera and increased <i>Intestinimonas</i> and <i>Coprobacter</i> .	Wang <i>et al</i> (2019)
Male CD-1 mice	Bisphenol A (BPA)	The family <i>Helicobacteriaceae</i> , markedly increased. <i>Firmicutes</i> was reduced by high fat diet and BPA, and this fall was in the class <i>Clostridia</i>	Lai <i>et al</i> , (2016)
Female California mice	Bisphenol A (BPA) (50 mg/kg feed)	Increases in <i>Bacteroides</i> , <i>Mollicutes</i> , <i>Prevotellaceae</i> and <i>Sutterella</i>) in males and females in parents. Increase in <i>Bifidobacterium</i> in F1 females	Javurek <i>et al</i> (2016)
ICR mice	Polystyrene microplastic (MP) particles (oral exposure to 1000 µg/L of 0.5 and 50 µm polystyrene MP)	Reduced the caecal abundance of <i>Firmicutes</i> , <i>Actinobacteria</i> and α - <i>Proteobacteria</i> , with a downward trend in <i>Bacteroidetes</i> ,. increase in <i>Proteobacteria</i> and <i>Actinobacteria</i> by 16S rRNA gene sequencing	Lu <i>et al</i> (2018)
Male C57BL/6 mice	The ionic liquid 1 octyl 3 methylimidazolium chloride [C8mim, M8OI] (440 mg/l in drinking water for 18 weeks)	Reduced relative abundance of <i>Parasutterella</i> , greater relative abundance of genera belonging to the <i>Lachnospiraceae</i> . <i>Coriobacteriaceae</i> spp. significantly increased.	Young <i>et al.</i> , 2020
Male C57BL/6 mice	The ionic liquid 1 butyl 3 methylimidazolium chloride [C4mim, BMI] (440 mg/l in drinking water for 18 weeks)	Reduced relative abundance of <i>Parasutterella</i> , greater relative abundance of genera belonging to the <i>Lachnospiraceae</i> . <i>Ruminococcaceae</i> exhibited a 25.64-fold decrease following BMI exposure.	Young <i>et al.</i> , 2020