TOX/2020/25

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

Toxicological interactions between xenobiotics and the human microbiota - Second draft statement

Introduction

1. At the March 2019 meeting, in horizon scanning, the COT expressed a wish to review the data on the effect of xenobiotics on the gastrointestinal microbiota and the effects of the microbiota on ingested xenobiotics and how these factors may need to be taken into account, if and as necessary, in risk assessment.

2. In November 2019, a scoping paper was prepared and presented to the Committee, covering recent work documented in the literature on the effects of metals, pesticides, food contact materials, ethanol, artificial sweeteners, environmental pollutants, antibiotics and pharmaceutical, and mycotoxins on the community structure of the gut microbiota of largely experimental animals with some human examples. In addition, the action of the gut microbiota on xenobiotics was documented, as well as how xenobiotic influences on the gut microbiota could be taken into account in chemical risk assessment.

3. In January 2020, a first draft statement was prepared and presented to the Committee, following comments made on the scoping paper, concentrating more on human studies. The committee decided that it would like to see more information on the functional capacity of the microbiota (paragraphs 26 – 35), effects on antimicrobial resistance)included in paragraphs 65 – 73), the intestinal barrier and immune response (mentioned throughout the text and paragraphs 33 – 35) and the non-bacterial components of the flora (paragraphs 36 – 48).

4. The Committee agreed for a statement to be prepared as an overview of the current state of knowledge in this area, with an emphasis on relevance to humans. It would need to highlight where the knowledge gaps were and critically address the extent to which the literature might apply to the work of the COT. The second draft statement is attached at Annex A.

Questions for the committee

5. Does the Committee have any comments on the structure and content of this second draft Statement?

Secretariat

May 2020

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

Toxicological interactions between xenobiotics and the human microbiota - First draft statement

Introduction

1 The term human microbiota refers to the population of microorganisms (bacteria, viruses, fungi, archea, 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 microbiota[1] 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 with particular emphasis on the human gut.

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 majority of the internal and external compartments of the human body are inhabited by microorganisms. By far the greatest number and variety of microorganisms is 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 and from individual to individual and depends upon diet (David et al, 2014) and locality. This statement concentrates on the bacteria 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 this Statement as well as in the literature.

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 are that 5,000 to 10,000 species, or perhaps many more may be represented, although an individual may harbour many fewer species than this. The gene set of the gut

[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).

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 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 data on the microbiome in relation to health and disease. Transcriptomics, proteomics and metabolomics have 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 found that by using a variety of culture conditions they could identify the presence of taxa that could not be identified by any single method alone. Moreover, 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 that related to essential functions of the bacteria and those that did 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. (Milari et al, 2017). 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. Children have a more diverse gut population than adults, probably because diet preferences and lifestyle are still being established and habitual diets tend to decrease microbiota diversity (Heiman and Greenway, 2016).

8 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.

9 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 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 that an earlier study by Schwiertz et al (2010), who found that a reduction in the F/B ratio was associated with 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.

10 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. Lifestyle factors showed much greater correlations with bacterial diversity than did 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 of host genetic factors such as SNPs with microbiome composition, however, the authors recommended using shotgun metagenomics instead of 16S rRNA profiling and pooling large data sets.

Metabolites produced by gut microbiota

Short Chain Fatty Acids (SCFAs)

11 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, 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.

12 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 PIP_3. 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.

13 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 seems to improve various neurological conditions, such as Parkinson's disease, depression and schizophrenia. Hoyles et al (2018) found 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 were concentration-dependent since high concentrations, especially of propionate, had been associated with the expression of autism-related genes.

14 Acetate has also been found to mediate intestinal IgA release via activation of GRP43 receptors. This effect was not mimicked by butyrate and was independent of T cells. Acetate did not promote IgA production by directly stimulating B cells but by activating retinoic acid production of regulatory dendritic cells, which then induced B cells to produce IgA.

15 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 SCFA 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".

16 SCFAs thus appear to be multifunctional effectors linking the metabolism of the gut microbiota to host physiology.

Bile acids

17 The steroidal metabolites of cholesterol conjugated with glycine (in humans) or taurine (in rats) that are produced by the liver and stored in the gall bladder 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

18 The gut microbiota are capable of synthesising 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.

19 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 ligands and effectors in inflammatory bowel disease.

20 Anaerobic choline metabolism by the microbiota produces trimethylamine (TMA), acetate and ethanol. Dysbiosis leading to aberrant choline metabolism has been proposed as potential contributing factor in non-alcoholic fatty liver disease, and increased TMA in circulation has been mooted as a risk factor for cardiovascular disease and colon cancer. The choline utilization (cut) gene cluster in sulphate 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

21 Prebiotics are foods or components in foods that are supposed 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).

22 Probiotics are 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 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.

23 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. Outcomes showed improvements in cases of obesity, insulin resistance and type 2 diabetes.

24 However, the effects of interventions to elucidate 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 animals differ. Nguyen et al (2015) considered how informative the use of mouse models is in relation to research on human gut microbiota. The authors considered the effects of anatomical, environmental and dietary factors on the composition of mouse gut microbiota and the apparent differences from 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 applied to 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 and the animals are in an intrinsically morbid condition.

25 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 the effect of xenobiotics and their metabolites is even more uncertain.

Functional aspects of the human gut flora

26 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 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.

27 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.

28 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 impact its functional capacities. Bacterial communities in the digestive tract in general were much more functionally robust in the face of taxonomic shifts than those from other body sites such as the skin, nostrils and vagina.

29 Gutierrez and Garrido (2019) performed a study whereby they 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 and 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.

30 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 shows 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 archeal 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.

31 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."

32 Nichols et al (2018) have produced a "hands-on" guide for toxicologists wishing to study the structure and function of the gut microbiome.

Intestinal immune response

33 Walker (2014) concisely reviewed the relationship between the colonising bacteria of the gut and the innate and adaptive immune systems in preventing pathogen invasion. Commensal bacteria stimulate the release of mucus from goblet cells in the intestinal epithelium as well as the expression of tight junction proteins between epithelial cells, providing protection against the penetration of large molecules and microbiota. The synthesis of antimicrobial molecules (defensins) by the epithelial cells is also stimulated. Notwithstanding the tight junctions, dendritic cells underlying the epithelium have been found to extend processes between epithelial cells and express Toll-like receptors (TLRs) in the gut lumen. Interaction of bacterial surface proteins with these TLRs leads to cytokine release that promotes the differentiation of T-helper cells and maintains gut innate immune homeostasis. 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.

34 As animals and people age, the immune functions of the gut appear to degrade, 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 lipopolysaccharide (LPS) producing species and a decrease in Firmicutes species (reviewed by Takiishi et al, 2017).

35 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 out 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.

Non-bacterial components of the gut microbiota

Fungi

36 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, gender, 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 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.

37 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 gender, 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% 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.

38 Schei et al (2017) investigated the possibility that, similar to the 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 possibly had their source in breast milk and yet others such as those of the Agaricomycetes, were recognised as being of environmental origin.

39 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)). Correlations have been made between changes in 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 possibly neurological diseases such as multiple sclerosis and motor neurone disease (Forbes et al, 2019).

40 Forbes et al (2019) published a parallel 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 stepwise increase up to the last plotted measurement, again in 2018, of about 27 papers per year

Viruses

41 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.

42 Manriqué et al (2016) discovered a healthy human phageome consisting of bacteriophages that 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 in a population and a third group that had some overlap between individuals. 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. The phage population appears to play a critical role in maintaining the ecosystem. in the murine gut and the authors presumed that the relationship would be similar in the human gut

43 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), Garmaeva et al (2019), Zuo et al (2019), Lawrence et al (2019), Mukhopadhya et al (2019), Neil et al (2018), Carding et al (2017), Lim et al (2015), Ogilvie & Jones (2015), Minot et al (2011))

Archea

44 The human gut (along with other niches) also plays host to members of the Archea, 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 archea and the gut bacteria, but no unequivocal correlations have been seen between the arches 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

45 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. The presence of intestinal worms, conversely, is regarded as largely beneficial for the immune system of the host and has been associated with a reduction in autoimmune diseases such as allergies, atopic dermatitis and asthma.

46 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 present at estimated frequencies of 0% in Italy and the USA, but increasing in non-industrialised populations reaching up to 90% in Peruvian hunter-gatherers.

47 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 mouse gut changes the expression of proteins in the mucosal layer, switching from MUC2 to MUC5AC and promotes the worm's egestion.

48 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.

Effect of xenobiotics on the gut microbiota

49 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 Table 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 whether an effect can be caused, which may not be possible or ethical to show with humans. The findings are briefly summarised below:

50 In the animal studies, xenobiotics of all types affected 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 genera to different degrees.

51 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 specific 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.

52 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 to best leave the gut flora unaffected, i.e. if it is not excreted in bile then a parenteral route could be used.

53 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 and BPA. Different studies found a variety of taxonomic changes, sometimes with increases in potentially inflammatory conditions and decrease in "beneficial" bacteria, although this was not universally the case.

54 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 reduced, Aspergillus spp and others increased) that were concomitant with increased severity of DSS-induced colitis and house-dust-mite-extract-induced allergic airway disease.

55 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. This might indicate 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 toxic or adaptive. There is also widespread extrapolation of results to the human condition.

Human in vivo and in vitro studies

Metals

56 Dong et al (2017) investigated the effects of arsenic (As) in drinking water on the intestinal microbiota of Bangladeshi children. High arsenic concentrations (218.8 ± 166.1 μg/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 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.

57 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.

58 Yin et al (2019) reported the production of silver nanoparticles 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 produce by gut microbiota, with unknown consequences for microbial and host health.

59 Cattò et al (2019) studied the interactions between non-lethal concentrations of citrate-capped silver nano-particles (AgNP) (1 μg/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 Megasphoem genus, which has been associated with antibiotic resistance and stress response. At the species level, falls were noted in the titres of Faecalbacterium prausnitzii and Clostridium coccides/ Eubacterium rectales, a condition seen in patients with intestinal inflammation and ulcerative colitis. No treatment affected SCFA production, but AgNP-BS markedly counteracted functional changes induced by AgNP alone treatment, particularly in the microbiota's capacity for xenobiotic degradation and metabolism.

60 Meyer et al (2008) reviewed papers that showed that methanoarchaea isolated from the human gut (Methanosphaera stadtmanae, Methanobrevibacter smithii) showed a higher potential for metal and metalloid (arsenic, antimony, bismuth, selenium, tellurium and mercury) derivatisation 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. Trimethylbismuth ($(CH_3)_3Bi$), 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 direct interaction with host cells and by disturbing the gut microflora.

Pesticides

61 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 might affect health in very young infants.

62 Schneeberger et al (2018) treated hookworm-positive adolescents aged 15 to 18 years from Cote d'Ivoire with four anthelminthic drug regimens (tribendimidine ± ivermectin, tribendimidine ± oxantel pamoate and albendazole ± oxantel pamoate for three weeks. On treatment arm 2, the titre of Bacteroidetes in stool samples was increased at 24 hours but had reduced to baseline by the end of treatment. Increases were noted for biotin metabolism, folate synthesis and N-glycan biosynthesis.

Antibiotics

63 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 using the commonly used 16S rRNA genetic analysis since this technique detects all bacteria in a population, including those that are dead, dormant and 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).

64 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 Megasphera and Veillonella) and large increases in the Proteobacteria and the Fusobacteria. Recovery from antibiotic varied between individuals and the rate of recovery was associated with infection with pathogens such as Klebsiella, Escherichia and Shigella.

65 Of 102 patients treated with antibiotics, Meletiadis 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.

66 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 their own 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.

67 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 either loss of membrane integrity (Pi*) or loss of membrane polarity ($DiBAC^+$). The HNA/ high energy phenotype correlated with the highest level of damage and was narrowed down 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.

68 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 falls 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).

69 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 the MFX were measured over the treatment period. DAV132 treatment had no significant effect in the blood pharmacokinetics of the MFX but significantly reduced the MFX AUC of the faecal pharmacokinetics. 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 cephalospirins, carbopenems, fluoroquinolones and the lincosamide clincamycin, 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.

70 Nogacka et al (2017) studied the effects on the intestinal microbiota and the presence of a range of AMR genes in vaginally delivered neonates in the absence and presence of maternal antibiotic prophylaxis during the intrapartum period. 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.

71 In the above study, IAP caused a slight but non-significant delay in SCFA production as seen up to 10 days but thereafter no 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 at 10 and 90 days. 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 !AP, 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 decided 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.

72 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. The authors made the observation that although the appropriate use of antibiotics saves lives, their use in pregnancy and infancy should be critically assessed in the light of potential consequences later in life.

73 Sarmiento et al (2019) correlated 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 industrial 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, quinolines, sulphonamides, macrolides and efflux pumps. A total of 17 AMR genes were found in normal, overweight and obese individuals, 2 were shared between normal and obese, 3 between overweight and obese and 4 and 1 were unique to 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

74 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 sulphur-containing amino acids, putative neurotransmitters, intermediates of energy metabolism and oxidative stress products. Possible mechanisms of action within the cocktail of compounds were recognised as diverse, including acting as surrogate electron acceptors, upregulating microbial GABA production and increasing angiogenesis. 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.

75 Montassier et al (2017) investigated the effect of chemotherapy with a cocktail of agents (bis-chloroethylnitrourea, etopside, 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 epithelial mucus layer may have been reduced and was suggested as a possible linkage between the known condition of chemotherapy-induced Gi mucocitis and dysbiosis.

76 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) and anticholinergic drugs reduced Bifidobacterium levels and increased the Lachnospiraceae. PD-induced depletion in the latter family is correlated with a decrease in SCFA production, with its 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.

77 Minalyan et al (2017) reviewed the effects of protein pump inhibitors 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 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.

78 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 20μM 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.

79 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 that 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.

80 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,

81 Maier et al (2017) fed human adults (26 women and 13 men) with reduced insulin sensitivity on 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 high-resistance starch. The high resistance starch 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.

82 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 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 function and thence of the gut-brain axis.

83 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 effects of xenobiotics.

Sweeteners

84 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 OD_{600}, 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. 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.

85 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 a1-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 papers on the microbiome to suggest that they raised safety concerns at their currently applied levels.

Environmental Pollutants

86 Saint-Cyr et al (2013) Assessed the effects of No-Observed-Adverse Effect-Level doses of deoxynivalenol (DON) on human gut flora transplanted into germ-free male Sprague Dawley rats. After allowing the faecal transplants 2 weeks to stabilise, rats were administered DON at 100 μg/kg bw by gavage for 4 weeks. Faecal samples were collected weekly up to day 27 and then on day 37 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 since DON at its toxicological NOAEL had effects on the gut microbiota with unknown physiological effects the approach of establishing a microbiological NOAEL for veterinary drugs should be considered for mycotoxins

87 The environment contains a rich variety of chemical entities that may enter the digestive tract of an animal and have toxicological consequences either directly or by affecting the composition and/ or functioning of commensal organisms. Inhaled PM2.5 and PM10 particles from natural (forest fires, volcanoes) and man-made (vehicle exhausts and smoking) may be delivered to the digestive tract from the lungs by the action of the mucocilliary escalator system in the trachea. Direct effects on the growth of gut microbiota or metabolic disturbances may then ensue from the presence of metals and organic components, such as PAHs, leading to the development of inflammation-associated conditions (Salim et al 2013).

88 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 DNA-reactive 7-hydroxy derivative and can deconjugate the hepatic product of phase 2 metabolism of BaP back to the parent compound. PCBs can be methylsulphonated 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.

89 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.

90 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 changes a range of volatile compounds produced by the bacteria (the "volatolome") caused by the BaP 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.

91 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 exposure in total up-regulated 613 genes and down-regulated 419 genes.

92 Gnotobiotic (germ-free) 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 mg/kg bw or sesame oil vehicle. 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

93 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 added directly to foods have so far been insufficiently studied for any statement on their effects to be made. The polymer chitosan is known to be antimicrobial (Gram+ > Gram -) and has been shown in pigs and humans to reduce the Firmicutes / Bacteroidetes ratio. Other compounds found 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.

94 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 degrade relatively innocuous chemicals to active products. For example, sulphates and sulphur-containing amino acids in the diet may be reduced to hydrogen sulphide, which may lead to reduced functioning of colonic epithelial cells, inflammation and increased rates of colon cancer. Nitrate reduction results in the production of 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).

95 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 mercuryin closed off loops of rat intestine and in the rumen of red deer could lead to methylmercury (MeHg) production. Incubation of $HgCl_2$ 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 polysulphides rather than MeHg, suggesting that the nature of the substrate can affect species transformation and hence toxicity.

96 Methylmercury demethylation is a function of which the rat and marine fish gut biota are capable and one which confers protection against the neurotoxic effect of organic mercury in the diet. Guo et al (2018) tested the effect of methyl mercury 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 to demethylate methylmercury and 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.

97 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 discovered in literature searches:

98 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.

99 Li and Jia (2013) listed the metabolic actions of microbiota-expressed enzymes on a range of drugs. Activities included reduction, hydrolysis, diacylation, 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.

100 Kim (2015) covered a range of drugs and concentrates on mechanisms activation by microbial metabolism. Mechanisms covered are azo, nitro, sulphoxide, N-oxide, C=C, O-N and C-N reduction, deglycosylation, ring fission, desulphation, deamination, hydroxylation and dihydroxylation. Antibiotics potentiated some effects and antagonised others, although the mechanism behind this difference was not discussed.

101 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.

102 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-sulphation 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, nitroreduction of nitrazepam leading to teratogenicity by Clostridium leptum and increased activity of the anthelmintic levanosole by thiazole ring opening caused by Bacteroides and Clostridium spp.

103 The metabolite p-cresol derived from the protein amino acid tyrosine, appears to compete for the same microbiotic O-sulphation pathway as paracetamol. 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)

104 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.

Plasma AChE activity was unaffected in the pesticide workers. BALB/c mice were administered MCP orally in drinking water at 28 μg/kg bw (10x 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. Acetate produced by microbial metabolism of the OP was found to induce gluconeogenesis, and a trend for higher acetate levels was found in the faeces of the diabetic OP workers.

105 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 (germ-free) 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.

106 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 exocytic amino group of HAAs and is thought to reduce their ability to bind DNA.

Statins

107 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 coprestanol, 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.

108 The statin lovastatin is a prodrug that requires hydrolysis to its β-hydroxy metabolite to be activated to its HMG-CoA reductase-inhibitory form. 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 co-administered. an antibiotic would have a reduced level of the activated metabolite and hence reduced effectiveness of lovastatin.

Probiotics

109 Unno et al (2015) fed six healthy female volunteers 2 servings daily for three weeks of a fermented milk product containing Lactobacillus acidophilus, Lactobacillus brevis, Bifidobacterium longum, Lactobacillus casei and Streptococcus thermphilus. 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.

110 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 bacterium 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.

111 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 x 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

112 Considering the multitude of interactions between the gut microbiota and chemicals of various classes ingested with food, Licht and Bahl (2018) considered 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 differs and overlaps 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.

113 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 a whole animal. Single bacteria or whole communities could also be assessed by “gut on a chip” microfluidic technology.

<u>Derivation of microbiological health-based guidance values</u>

114 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 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).

115 In their 2018 report, the Joint Food and Agriculture Organization. World Health Organization Meeting on Pesticide Residues (JMPR) 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…" Disruption in the composition of the intestinal microbiome, including the fungal communities, by residues of fungicides or by other pesticides could have an impact on intestinal homeostasis and systemic immunity. In 2017, JMPR therefore recommended that studies of the effects of pesticides on the intestinal microbiota should be routinely considered, following the step-wise decision-tree approach used by JECFA when establishing a microbiological ADI and ARfD for veterinary drugs.

116 The 85th JECFA 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).

117 Points to be considered before a microbiological HBGV for a particular xenobiotic (as applied by JECFA to 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?

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

119 The calculations for microbiological ADIs and ARfD 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:

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

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 MIC_{50} (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.

Body weight = 60 kg.

120 JECFA (2018) state 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."

Conclusions

121 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.

122 Investigations into the non-bacterial components of the gut microbiota (fungi, viruses, protozoa and archea) are documented 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.

123 The majority of investigations into the effect of xenobiotics on the gut microbiota have used animal models, the data from which have been extrapolated 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.

124 From animal studies it is known 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 only address changes in the more tractable part of the bacterial community and still give an incomplete estimate of changes as a whole.

125 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 to the host or adaptive in nature.

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

127 A wide range of substances can influence the species balance of the gut microbiota to the extent that almost anything ingested affects structure and function (but see below).

128 Despite sometimes large-scale variability in the composition of 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 equivalent 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.

129 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.

130 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.

131 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.

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	
Coccidiostat	
Fungicide	
Aldrin	
Dieldrin	
Flame retardant	
Metabolite	

Some references were found in the reference lists of papers acquired in the PubMed searches.

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	*Bacillus cereus*
BDE	Brominated diphenylether
BPA	Bisphenol A
BS	*Bacillus subtilis*
CCAAT	A DNA transcription initiation site
Cd	Cadmium
C/ERPα	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	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DOM-1	Deepoxydeoxynivalenol
DON	Deoxynivalenol
DONGlc	Deoxynivalenol-3-glucoside
DSS	Dextran sulphate sodium
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 sulphate
FXR	Farnesyl-X receptor
GC	Gas chromatography
GI	Gastrointestinal
GPR	Orphan G-protein-coupled receptor
HAA	Heterocyclic aromatic amines
HBCDD	Hexabromocyclododecane
HDL	High-density lipoproteins
Hg	Mercury
HgS	Mercuric sulphide, 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
IgA	Immunoglobulin A
IQ	2-amino-3-methylimidazo[4,5-f]quinoline
JECFA	Joint FAO/WHO Expert Committee on Food Additives
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 flavinoid signalling receptor
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 sulphonic 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)
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 *Desulfovibrio*, increase in anti-inflammatory *Bifidobacterium.*	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 *Bacteroidetes* and *Firmicutes,* increased *Proteobacteria* and *Actinobacteria*	Mahalal et al (2018)
ICR mice	Iron plus arsenic	Increase in *Firmicutes,* decrease in *Bacteroidetes* and *Actinobacteria*.	Guo et al (2014)
C57BL/6 mice	Arsenic	Increase in *Bacteroidia,* decrease in *Clostridia classes* with minor classes growing or receding	Dheer et al (2014)
C57BL/6 mice		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	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	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	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.	Fazelli et al (2011)
Balb/c mice		Reduced *Firmicutes / Bacteroidetes* ratio, and population of *Lactobacilli* and *Bifidobacteria.* Intestinal mucus layer thinning, increased colon TNFα , reduced production of SCFA	Liu et al (2014)
C57BL/6 mice		Fat mass increased and microbial diversity decreased. Plasma triglycerides, total cholesterol, free FA, leptin HDL and liver triglycerides increased. *Bacteroidetes* increased and *Firmicutes* decreased.	Ba et al (2017)
Balb/c mice		No effect on bacterial diversity by 16S rRNA analysis in the caecum and faeces of female Balc/c mice. The relative proportions of different families and genera markedly affected	Breton et al (2013a)
Germ-free and SPF C57BL/6	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)

mice			

C57BL/6 mice	Lead	*Bacteroidetes* decreased and *Firmicutes* increased. Fewer culturable aerobes and more culturable anaerobes in the faeces	Wu et al (2016)
C57BL/6 mice		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 *Firmicutes* were reduced by <0.1 mg/l lead whereas *Bacteroidetes* reduced only at 0.1 mg/l. *Proteobacteria* and *Actinobacteria* 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 *Firmicutes*/*Bacteroidetes* 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: *Parabacterioides*, *Lactobacilli* and *Allobaculum*) increased, *Aldercreutzia* and unclassified *Clostridiaceae*) decreased. Acetate production decreased, trimethylamine increased. Biofilm production increased	Pinget et al (2019)
Mice	Silver (NP)	Increased ileal *Firmicutes/Bacteroidetes* ratio, Balance of genera changed Older NP less effective possibly due to sulphidation	Van den Brule et al (2016)
Sprague Dawley rats		*Bacteroidetes* and *Firmicutes* 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		No significant changes	Wilding et al (2016)
SPF Balb/c mice	Gold (NP)	Increase in *Proteobacteria.* (*Roseburia* were depleted and *Staphyllococcus, Ureoplasma* and *Methylobacterium* 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 -HCH	Reduced *Actinobacteria* and the *Candidatus Saccharibacteria* no effect on *Bacterioidetes, Firmicutes, Verrucomicrobia* or *Proteobacteria*.	Liu *et al* (2017)
Male ICR mice	Endosulfan	Serum hippurate levels fell dimethylalanine and trimethylamine N-oxide increased.	Zhang *et al* (2017)
Wistar rats	Permethrin (75:25 trans:cis)	Various *Bacteroidetes Lactobacillus* spp temporarily increased *Bacteroidetes* spp were significantly reduced	Nasuti *et al* (2016)
Mice	Chlorpyrifos	Chlorpyrifos treatment of mice on a normal diet had symptoms similar to those on the high fat diet.	Liang *et al* (2019
Wistar rats		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 *et al* (2018)
Wistar rats		Reduced *Firmicutes* and other SCFA-producing bacteria partially reversed by inulin	Reygner, Lichtenburger, *et al* (2016)
SPF C57BL/6 mice	Diazinon	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 *et al* (2017)
SPF C57BL/6 mice	Malathion	Genes for quorum sensing flagellar proteins pathogenicity and virulence. upregulated	Gao *et al* (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 *et al* (2019)
Male Sp rague Dawley rats	Glyphosate	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 *et al* (2018)
Sprague Dawley rats		*Bacteroidetes* family S24-7 increased. *Lactobacilliaceae* decreased in 8 out of 9 treated animals. In vitro, *Bifidobacteria, Clostridia* and *Enterococci* were sensitive to glyphosate at 400 ppm, *Lactobacilli* above 5000 ppm and coliforms not sensitive	Loranzo *et al* (2018)
Male Swiss mice		*Firmicutes, Corynebacterium, Bacteroidetes* spp and *Lactobacillus* spp depleted.	Aitbali *et al* (2018)

Sprague Dawley rats		Significant changes in the balance of the microbiota in the glyphosate- and Roundup™-treated dams and pups compared with a water control	Mao *et al* (2018)
ICR and C57Bl/6 mice	Imazalil	Fall in the relative abundance of the *Bacteroidetes* at all imazilil doses and in the *Firmicutes* and *Actinobacteria* at the highest dose and later time points; increases on *Firmicutes, α-Proteobacteria* and *γ-Proteobacteria*	Jun *et al* (2016 and 2018)
C57Bl/6 mice	fluconazole	Decrease in *Candida spp*, increase in *Aspergillus, Wallemia* and *Epicoccum* exacerbation of DSS-induced colitis and house-dust-mite-extract-induced allergic airway disease	Wheeler *et al* (2016)
ICR mice	propamocarb	*Bacteroidetes* α-Proteobacteria and γ-Proteobacteria reduced *Firmicutes* increased at 3 mg/l and then fell. In the caecum the *Actinobacteria* and *β-Proteobacteria* fell but little change in the other phyla at 300 mg/l	Wu *et al* (2018a)
ICR mice	propamocarb	Faecal expression of genes involved with bile acid synthesis and transport increased. trimethylamine levels were increased	Wu *et al* (2018b)
Female Sprague Dawley rats	epoxiconazole	Increasing *Bacteroidetes* and the *Proteobacteria* decrease in *Firmicutes*	Xu *et al* (2014)
SPF C57BL/6 mice	2,4-dichlorophenoxyacetic acid (2,4-D	Increased *Bacteroidetes, Chlorobi, Chloroflexi, Spirochetes* and *Thermotogae* no change in *Acidobacteria*,	Tu *et al* (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 and tetracycline	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 *et al* (2013)
Pregnant C57 Bl/6 mice	Penicillin V	A*ctinobacteria* decreased, *Bacteroidetes and Firmicutes* both increased	Leclercq *et al* (2016)
Male and female Wistar rats	4-EOTC, a major oxytetracycline metabolite	Fall in *Bacteroidetes. Actinomycetes (Bifidobacteria) increased*. The families *Lactobacilliaceae (Helicobacteriaceae* depleted. Tetracycline resistance increased	Han *et al* (2016)
Male Sprague Dawley rats	Ampicillin, neomycin, gentamicin, metronidazole and vancomycin, then mixed atrazine, simazine, ametryn, terbuthylazine and metribuzin.	Increased bioavailability of all the triazines without affecting microbial or hepatic triazine metabolism.	Zhan *et al* (2018)
Weanling C57BL6 mice	Penicillin, vancomycin, penicillin-plus-vancomycin or chlortetracycline for 7 weeks, in drinking water	*Lachnospiriaceae* family increased, *F/ B* ratio increased. Butyryl CoA transferase copy number decreased at 3 weeks but increased, along with SCFAs by 6 weeks,	Cho *et al* (2012)

Female BALB/c mice	Ciprofloxacin (cip) and metronidazole or vancomycin in drinking water for 3 weeks	*Bacteroidetes* and *Actinobacteria* depleted, *Proteobacteria* increased. *F/B* increased. *Streptococcaceae* increased with cip, *Lactobacillaceae*	Nagano *et al* (2019)

		and *Enterobacteraceae* increased with vancomycin	

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

Animals	Xenobiotic	Effect	Reference
C57BL/6 mice	Morphine, slow release pellets	Reduced *Bacteroidetes.* In the *Firmicutes*, *Enterococcaceae, Staphyllococcaceae, Bacillaceae, Streptococcaceae* and *Erysipelotrichaceae* increased. Gut barrier negatively affected and bacteria (mainly Gram positive) translocated through the epithelium	Banarjee *et al* (2016)
Male C57BL6 mice	Carboxymethylcellulose (CMC) or polysorbate-80 (P80)	*Firmicutes* decreased, *Bacteroidetes* increased, making conditions more pro- inflammatory.	Viennois *et al* (2017)
Female C57BL/6 N mice	Soy or coconut oil	Coconut-oil-fed mice had higher blood cholesterol level after 8 weeks. and a greater relative abundance of *Allobaculum* and *Anaerofustis* and depletion of *Akkermansia* compared with soy oil.	Patrone *et al* (2018)
Male and female CD-1 mice	Acesulfame-K	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 *et al* (2017)
Mouse model of Cohn's disease	Splenda (1% sucralose, 99% maltodextrin w/w)	Over growth of 5 classes within the phylum *Proteobacteria*. No significant change to *Firmicutes* or *Bacteroidetes*.	Rodriguez-Palacios *et al* (2018)
Male C57BL6 mice	Sucralose	Genes for LPS synthesis and flagellar components and fimbriae were up-regulated	Biam *et al* (2017)
Male C57BL/6 mice	Xylitol	No significant changes	Uebanso *et al* (2017)

Male Sprague Dawley rats	Ethanol	Significant changes ($p<0.05$) to diversity, richness and evenness of the colonic	Mutlu *et al* (2009)

		population Addition of oats or the probiotic Lactobacillus, maintained parameters at control levels	
Male Sprague Dawley rats		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	Xie *et al* (2013)
Male C57BL/6 mice		Reduced *Firmicutes* and *Bacteroidetes* Over growth of the *Actinobacteria* and *Proteobacteria*	Bull-Otterson *et al* (2013)
Female C57BL/6 mice		No changes in bacterial diversity but an increase in the *Actinobacteria* and a decrease in *Verrucomicrobia*	Lowe *et al* (2017) and Lowe *et al* (2018)
Male Sprague Dawley rats	Aflatoxin B1 (AFB1)	Slight reduction in *Bacteroidetes* minor increases in *Firmicutes* and *Proteobacteria* but marked change at the genus level.	Liew *et al* (2019)
Male F344 rats		Reduced genetic diversity No major phylum level shifts but *Firmicutes* species were unchanged or increased, but lactic acid bacteria, reduced, Bacteroidetes unchanged or slightly reduced	Wang *et al* (2015)
Male C57BL/6 mice	BaP	Faecal *Bacteroides* increased and *Verrucomicrobia* decreased. *Lactobacillus* and *Akkermansia*, decreased in t faeces. Different mucosal taxa increased and decreased, depending upon location.	Ribière *et al* (2016)
Male C57BL/6 mice	2,3,7,8-tetrachlorodibenzo-*p*- dioxin (TCDD).	Increased relative abundance of species in the genera *Lactobacillus, Clostridium, Streptococcus* and *Listeria*.	Fader *et al* (2017)
C57BL/6 mice (*Ahr* $^{-/-}$ and *Ahr* $^{+/+}$)	2,3,7,8-tetrachloribenzofuran (TCDF)	Decreased the *Firmicutes*/ *Bacteroidetes* ratio without affecting the *Actinobacteria.*	Zhang *et al* (2015)

Male *Ldtr*$^{-/-}$ mice	Polychlorinated biphenyl126 (PCB 126)	Reduced bacterial diversity with reductions in *Bifidobacterium, Lactobacillus* and *Ruminococcus* but an increase in *Akkermansia.* Inflammatory cytokines were increased,	(Petriello *et al,* 2018).

Female CD-1 mice	Perfluorooctane sulfonic acid (PFOS)	No effect on bacterial diversity. Some taxa increased and others decreased. Low-PFOS dose induced increase in the genus *Turicibacter*, The genus *Allobaculum*, a SCFA-producing genus also increased	Lai *et al* (2018)
Conventional and germ-free C57BL/6 mice	Polybrominated diphenyl ethers (PBDE),	Metabolism of PBDEs by mouse liver was modified by the presence or absence of gut microbiota	Li *et al* (2017)
Pregnant female ICR mice and their offspring	Triphenylphosphate (TPHP),	Increased bacterial classes *Erysilelotrichia* and *Bacilli* and decreased *Clostridia*. Genera *Allobaculum, Tunicibacter* and *Lactobacillus* increased.	Wang *et al* (2019)
Male C57BL/6 mice	Mono-2-ethylhexylphthalate ester (MEHP)	Increase in F*irmicutes*, reduced *Verrucmicrobia* and increase in the *Firmicutes*/*Bacteroidetes* ratio. Reduced *Akkermansial* and *Alloprevotella* genera and increased *Intestinimonas* and *Coprobacter*.	Wang *et al* (2019
Male CD-1 mice	Bisphenol A (BPA)	The family *Helicobacteriaceae*, markedly increased. *Firmicutes* was reduced by high fat diet and BPA, and this fall was in the class *Clostridia*	Lai *et al*, (2016)
Female California mice	Bisphenol A (BPA)	Increases in *Bacteroides, Mollicutes, Prevotellaceae* and *Sutterella*) in males and females in parents. Increase in *Bifidobacterium* in F1 females	Javurek *et al* (2016)
ICR mice	Polystyrene microplastic (MP) particles	Reduced the caecal abundance of *Firmicutes, Actinobacteria* and - *Proteobacteria,* with a downward trend in *Bacteroidetes*,. increase in *Proteobacteria* and Actinobacteria by 16S rRNA gene sequencing	Lu *et al* (2018)