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. 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.

3. 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.

4. The Committee concluded that the considerable inter-individual variation in the composition of the gut microbiota of human individuals made it difficult to extrapolate from effects on the flora of experimental animals to the human condition and recognised that the relationship between taxonomic changes and systemic diseases was correlative, not causative.

5. The Committee decided that long-term prospective studies across different age groups would be required to address effects of xenobiotics on the human microbiota. These studies would have to target specific parts of the GI tract since the composition of the flora varied naturally along its length. Another option would be to identify individual metabolic pathways that might be disrupted or specific microbial species, although this would reveal only a small part of the interactions that could potentially occur. A Member suggested that the later-life health consequences of caesarean section birth relative to vaginal birth could be amenable to research.

6. 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 draft statement

is attached at Annex A. In preparing the statement, two issues were identified that it would be helpful to have a steer on, so questions have been asked on those areas as well as requesting general information on the draft statement.

Questions for the committee

1 Do Members think that gnotobiotic animals inoculated with human gut microbiota may be used to give an indication of risk to humans posed by gastrointestinal exposure to xenobiotics?

2 Does the Committee think that, considering the apparent wide range of xenobiotics that can affect the balance of the microbiota, with unknown consequences on host health, that part of the safety assessment for new therapeutics should routinely include the possibility of setting a microbiological ADI?

3 Does the Committee have any comments on the structure and content of this draft Statement?

Secretariat

January 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

Composition and distribution of the microbiota

7. The term human microbiota refers to the population of microorganisms (bacteria, viruses, fungi, 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 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. There is a substantial body of literature on this topic, therefore this paper has used relatively narrow search terms and thus 9s a representative rather than comprehensive survey of the microbiota and their interaction with ingested xenobiotics.

8. Unless stated otherwise, the general information on the gut microbiota in this introduction (paragraphs 1 – 23) is taken from reviews by Rowland *et* al (2018), Jandhyala *et al* (2015) and Hollister *et al* (2014).

9. 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 majority 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 individual to individual and depends upon diet (David *et* al, 2014) and locality.

10. 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 microbiota – the 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

11. 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).

12. The composition of the gut microbiota of new-borns appears to be influenced 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). 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).

13. 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.

14. Changes in the ratio of *Firmicutes* to *Bacteroidetes* ratio are frequently used in the literature to indicate dysbiosis, possibly caused by an ingested substance. 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 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) 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.

Metabolites produced by gut microbiota

Short Chain Fatty Acids (SCFAs)

15. 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).

16. Kimura *et al* (2013) investigated the action of SCFAs at adipose tissue-expressed GPR43 G-protein-coupled receptors in wild type and *Gpr*43$^{-/-}$ 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 *Gpr*43$^{-/-}$ 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 was found to suppress 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.

17. Oleskin and Shenderov (2016) briefly reviewed observed effects of SCFAs on host neurotransmitter function. Propionate and butyrate appeared to regulate expression of the gene for tryptophan hydroxylase, the rate-limiting step in serotonin synthesis, and decrease the activity of histone deacetylases, which appears to improve various neurological conditions, such as Parkinson's disease, depression and schizophrenia. Such beneficial actions of SCFAs were concentration-dependent since high concentrations, especially of propionate, had been associated with the expression of autism-related genes.

18. 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.

19. 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".

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

Bile acids

21. The conjugated (glycine in humans, taurine in rats) steroidal metabolites of cholesterol 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

22. 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*.

23. 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.

24. 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, but the pathway may also have been acquired in some strains via horizontal gene transfer (Krishnan *et al,* 2015).

Prebiotics and probiotics

25. 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).

26. 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 this presence of that organism may itself be transient.

27. 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.

28. 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.

29. 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 has 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.

Effect of xenobiotics on the gut microbiota

30. 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:

31. In the animal studies, xenobiotics of all types affected the balance of the phyla in the gut.

32. Heavy metals such as arsenic, cadmium and lead tended to reduce the *F/B* ratio and lead to reduced SCFA production, 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.

33. 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.

34. 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.

35. A range of other compounds have been tested on the gut microbiota of rats and mice. These include 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.

36. The data from animal studies suggests 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, possibly indicating increased risk of growth of pathogens, reduction in the barrier function and health of the gut epithelium and thence systemic effects on the host. However, results vary between studies: what is observed for is not the same in all cases and 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

37. Dong *et al* (2017) investigated the effects of arsenic 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.

38. 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.

39. 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^{o}C for 48 hours. Spherical silver nanoparticles were observed under transmission electron microscopy on the surface and within microbial cells. The authors expressed concern that nanoparticles could be produce by gut microbiota, with unknown consequences for microbial and host health.

40. Cattò *et al* (2019) studied the interactions between non-lethal concentrations of citrate-capped 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.

Pesticides

41. Reygner, Condette *et al* (2016) used the SHIME model to study the direct effects of below-threshold 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.

42. 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 were concurrently increased.

Antibiotics

43. 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).

44. 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*.

45. 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.

46. 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, 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.

47. 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.

48. 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).

49. 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 celluloid 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 400μg/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.

Miscellaneous

50. 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.

51. 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.

52. 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 CNS.

53. 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 in 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 *Sterptococcaceae* and the *Micrococcaceae*. The changes observed were independent of antibiotic use although this was not associated with individual antibiotics or prolonged use. 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.

54. 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.

The effect of food components on the gut microbiota.

55. 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,

56. 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.

57. 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 and increased activity in the hypothalamic-pituitary-adrenal (HPA) axis and gut inflammation along with *Firmicutes/ Bacteroidetes* imbalance, and 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.

58. Shinohara *et al* (2010) observed that apple pectin consumption was associated with an improved intestinal environment because isolates of “beneficial” bacteria such as *Bifidibacteria* 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

59. 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.

60. 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 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 1-year exposure, indicating no microbial metabolic adaptation, even with very high doses. Neither saccharin nor acesulfame K underwent gastrointestinal metabolism, but, in contrast to sucralose, both of these latter 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.

Mycotoxins

61. 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

Environmental Pollutants

62. 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).

63. 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 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.

64. 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.

65. 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.

66. 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.

67. 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

68. 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.

69. 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)

70. Li *et al* (2019) reviewed the methylation and demethylation of mercury by the gut microbiota of fish, terrestrial invertebrates and mammals. Papers from as early as 1975 showed that anaerobic incubation of inorganic mercury in closed off loops of rat intestine and in the rumen of red deer could lead to methylmercury (MeHg) production. Incubation of Hg Cl_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*.

71. 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.

72. 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:

73. 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.

74. 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.

75. 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.

76. 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.

77. 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.

78. 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)

79. 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.

80. 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.

81. 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

82. 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.

83. 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

84. 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.

85. 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.

86. 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^{o}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

87. 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.

88. 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.

Derivation of microbiological health-based guidance values

89. 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).

90. 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).

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

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

92. 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.

93. 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:

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

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.

94. 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

95. 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

96. 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.

97. 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.

98. 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.

99. 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

100. 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.

101. 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.

102. 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.

103. 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

January 2020

References

Aitbali Y, BaM'hamed S, Elhider N, Nafis A, Soraa N. Glyphosate-based-herbicide exposure affects gut microbiota, anxiety and depression-like behaviours in mice. Neurotoxicology and Teratology 2018 **67**: 44 – 49.

Arat S, Spivak A, Van Horn S, Thomas E, Traini C, Sathe G, Livi GP, Ingraham K, Jones L, Aubert K, Ho;lmes DJ, narerer O, Brown JR. Microbiome changes in healthy volunteers treated with GSK1322322, a novel antibiotic targeting bacterial peptide deformylase. Antimicrobial Agents and Chemotherapy 2015 **59**(2): 1182 – 1192.

Ba O, Li M, Chan P, Huang C, Duan X, Lu L, Li J, Chu R, Xie D, Song H, Wu Y, Ying H, Jia X, Wang H. Sex-dependent effects if cadmium in early life on gut microbiota and fat accumulation in mice. Environmental Health Perspectives 2017 **123**(3): 437 – 446.

Banerjee S, Sindberg G, Wang F, Meng J, Sharma U, Zhang L, Dauer P, Chen C, Dalluge J, Johnson T, Roy S. Opioid-induced gut microbial disruption and bile dysregulation leads to gut barrier compromise and sustained systematic inflammation. Mucosal Immunology 2019 **8**(6): 1418 – 1428.

Beer F, Urbat F, Franz CMAP, Huch M, Kulling SE, Bunzel M, Bunzel D. The human fecal microbiota metabolizes foodborne heterocyclic aromatic amines by reuterin conjugation and further transformations. Molecular Nutrition and Food Research2019 **63**: doi: 10.1002/mnfr.2018117

Bian X, Chi L, Cao B, Tu P, Ru H, Lu K. The artificial sweetener aculfame potassium affects the gut microbiome and bodyweight gain in CD-1 mice. PLOS One 2017. https://doi.org/10.1371/journal.pone.0178426

Bian X, Chi L, Gao B, Tu P, Ru H, Lu K. Gut microbiome response to sucralose and its potential role in inducing liver inflammation in mice. Frontiers in Physiology 2017 **8**: 478 – 490.

Breton J, Daniel C, Dewulf J, Pothion S, Froux N, Sauty M, Thomas P, Pot B, Foligné B. Gut microbiota limits heavy metal burden caused by chronic oral exposure. Toxicology Letters 2013 **222**: 132 – 138.

Breton J, Massart S, Vandanne P, De Brandt E, Pot B, Foligné B. Ecotoxicology inside the gut: impact of heavy metals on the mouse microbiome. Pharmacology and Toxicology 2013 **14**: 64 – 74.

Bull-Otterson L, Feng W, Kirpich I, Wang Y, Qin X, Liu Y, Gobejishvili L, Joshi-barve S, Ayvaz T, Petrosino J, Kong M, Barker D, McClain C, Barve S. Metagenomic

analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus GG* treatment. PLOS One 2013 **8**(1) e53028.

Calatayud M, Xiong C, Du Laing G, Raber G, Francesconi K, van de Wiele T. Salivary and gut microbiomes play a significant role in *in vitro* oral bioaccessibility, biotransformation, and intestinal absorption of arsenic from food. Environmental Science and Technology 2018 **52** 14422 – 14435.

Cattò C, Garuglieri E, Borrusu L, Erba D, Casiraghi MC, Cappitelli F, Villa F, Zecchin S, Zanchi R. Impacts of sirtary silver nanoparticles and probiotic administration on the microbiota of an *in-vitro* gut model. Environmental Pollution 2019 **245**: 754 – 763.

Chi L, Bian X, Gao B, Ru H, Tu P, Lu K. Sex-specific effects of arsenic exposure on the trajectory and function of the gut microbiome. Chemical research in Toxicology 2016 **29**(6): 949 – 951.

Chi L, Gao B, Bian X, Tu P, Ru H, Lu K. Manganese-induced sex-specific gut microbiome perturbations in C57BL/6 mice. Toxicology and Applied Pharmacology 2017 **331**: 142 – 153.

Chi L, Xue J, Tu P, Lai Y, Ru H, Lu K. Gut microbiome disruption altered the biotransformation and liver toxicity of arsenic in mice. Archives of Toxicology 2019 **93**: 25 – 35.

Cho I, Yamanishi S, Cox L, Methé, BA, Zavadil J, Li K, Gao Z, Mahana D, Raju K, Teitier I, Li H, Alekseyenko AV, Blaser MJ. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012 **488**(7413): 621 - 626

Claus SP, GuillouH, Ellero-Simatos S. The gut microbiota: a major player in the toxicity of environmental pollutants? Biofilms and Microbiomes 2016**2** 16003; doi: 10.1038/npjbiogilms.2016.3.

Clayton TA, Baker D, Lindon JC, Everett JR, Nicholson JK. Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. PNAS 2009 **106**(34):14728 - 14733Constante M, Fragoso G, Lupien-Meilleur J, CalvéA, Santos MM. Iron supplements modulate colon microbiota composition and potentiate the protective effects of probiotics in dextran sodium sulfate-induced colitis. Inflammatory Bowel Diseases 2017 **24**: 758 – 766.

Costantini L, Molinari R, Farinon B, Merendino N. Impact of omega-3-fatty acids on the gut microbiota. International Journal of Molecular Sciences2017 **18**: 2645 – 2660.

Currò D. The role of gut microbiota in the modulation of drug action: a focus on some clinically significant issues. Expert Review of Clinical Pharmacology 2018 **11**(2): 171 - 183

David LA, Maurice CP, Carmody RN, Gootenberg DB, Button JE, Wolfa BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Buddinger SB, Dutton RJ, Turnbaugh PJ.

Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014 **505**(7484): 559 – 563.

Defois C, Ratel J, Denis S, Batut B, Beugnot R, Pevretaillade E, Engel E, Peyret P. Environmental pollutant benzo[a]pyrene impacts the volatile metabolome and transcriptome of the human gut microbiota. Frontiers of Microbiology 2017 **8**: 1563 – 1582.

Defois C, Ratel J, Gerrait G, Denis S, LeGoff O, Talvas J, Mosoni P, Engel E, Peyret P. Food chemicals disrupt human gut microbiota activity and impact intestinal homeostasis as revealed by *in vitro* systems. Nature Scientific Reports 2018 **8**:11006 doi: 10.1038/s41598-018-29376-9.

de Gunzburg J, Ghozlane A, Ducher A, Le Chatelier E, Duval X, Ruppé E, Armand-Lefevre L, Sablier-Gallis F, Burdet C, Alavoine L, Chachaty E, Augustin V, Varastet M, Levenez F, Kennedy S, Pons N, Mentré F, Andremont A. Protection of the human gut microbiome from antibiotics. The Journal of Infectious Diseases® 2018 **217**: 628–36.

Dheer R, Patterson J, Dudash M, Stachler EN, Bibby KJ, Stolz DB, Shiva S, Wang Z, Hazen SL, Barchowsky A, Stolz JF. Arsenic induces structural and compositional change and promotes nitrogen and amino acid metabolism. Toxicology and Applied Pharmacology 2015 **289**: 397 – 408.

Dong X, Shutzhenko N, Lemaitre J, Greer RL, Peremyslova K, Quamruzzaman Q, Rahman M, Sharif M, Hasan I, Joya SA, Golam M, Christiani DC, Morgan A, Kile ML. Arsenic exposure and intestinal microbiota in children from Sirajdikhan, Bangladesh. PLOS One 2017 12(12) e188487

Fader KA, Nault R, Zhang C, Kumagai K, harkema JR, Zacherowski TR. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-elicited effects on bile acid homeostasis; alterations in biosynthesis, enterohepatic circulation, and microbial metabolism. Nature Scientific Reports 2017 **7**: 5921 doi: 10.1038/s41598-017-05656-8.

Fang B, Li JW, Zhang M, Ren FZ, Pang GF. Chronic chlorpyrifos exposure elicits diet specific effects on metabolism and the gut microbiome in rats. Food and Chemical Toxicology 2018 **111**: 144 – 152.

Fazeli M, Hassanzadeh P, Alael S. cadmium chloride exhibits a profound toxic effect on the bacterial microflora of the mice gastrointestinal tract. Human and Experimental Toxicology 2011 **30**(2): 152 – 159.

Feng P, Ye Z, Kakade A, Virk AK, Li X, Liu P. A review on gut remediation of selected environmental contaminants: possible roles of probiotics and gut microbiota. Nutrients 2019 **11**:22 – 40.

Ferrer M, Mendez-Garcia C, Rojo D, Barbas C, Moya A. Antibiotic use and microbiome function. Biochemical Pharmacology 2017 **134**: 114 – 126.

Fijan S Microorganisms with claimed probiotic properties: an overview of recent literature. International Journal of Environmental Research and Public Health. 2014 **11**: 4745 – 4767.

Gao B, Bian X, Mahbub R, Lu K. Sex-specific effects of organophosphate diazinon on the gut microbiome and its metabolic functions. Environmental Health Perspectives **125**(2): 198 – 206.

Gao B, Chi L, Mahbub R, Bian X, Tu P, Ru H, Lu K. Multi-omics reveals that lead exposure disturbs gut microbiome development, key metabolites and metabolic pathways. Chemical Research in Toxicology 2017 **30**(4): 996 – 1005..

Gao B, Chi L, Tu P, Bian X, Thomas J, Ru H, Lu K. The organophosphate malathion disrupts gut microbiome development and the quorum-sensing system. Toxicology Letters 2018 **283**: 52 – 57.

Gao B, Chi L, Tu P, Gao N, Lu K. The carbamate aldicarb altered the gut microbiome, metabolome and lipidome of C57 BL/6 mice. Chemical Research in Toxicology 2019 **32**(1): 67 – 79.

Gaulke CA, Rolshoven J, Wong CP, Hudson LG, Ho E, Sharpton TJ. Marginal zinc deficiency and environmentally relevant concentrations of arsenic elicit combined effects on the gut microbiome. mSphere 2018 **3**(6): e00521-18 1 – 13

Gratz SW, Currie V, Richardson AL, Duncan G, Holtrop G, Fraquarson F, louis P, Pinton P, Oswald IP. Porcine small and large intestinal microbiota rapidly hydrolyse the masked mycotoxin deoxynivalenol-3-glucoside and release deoxynivalenol in spiked batch cultures *in vitro*. Applied and Environmental Microbiology 2018 **84**(2) e02106-17.

Groh KJ, Geneke B, Muncke J. Food contact materials and gut health: implications fortoxicity assessment and relevance of high molecular weight migrants. Food and Chemical Toxicology 2017 **109**: 1 – 18.

Guo G, Yumvihoze E, Poulain AJ, Chan HM. 2018 The Journal of Toxicological Sciences **43**(12): 717 – 725.

Guo X, Liu S, Wang Z, Zhang X, Li M, Wu B. Metagenomic profiles and antibiotic resistance genes in gut microbiota of mice exposed to arsenic and iron. Chemosphere 2014 **112**: 1 – 8.

Han H, Xiao H, Zhang K, Lu Z. Impact of 4-epi-oxytetracycline on the gut microbiota and blood metabolomics of Wistar rats. Nature Scientific Reports 2016 **6**L 23141 doi: 10.1038/srep23141

He X, Zheng N, He J, Liu C, Feng J, Jin W, Li H. Gut microbiota modulation attenuated the hypolipidemic effect of simvastatin in high-fat/cholesterol-diet fed mice. Journal of Proteome Research 2017 **16**(5): 1900 – 1910.

Heiman ML, Greenway FL. A healthy gastrointestinal microbiome is dependent on dietary diversity. Molecular Metabolism 2016 **9**: 317 - 320

Hiergeist A, Gläsner J, Reischl U, Gessner A. Analyses of intestinal microbiota: culture versus sequencing. Institute for Laboratory Animal Research Journal 2015 **56**(2): 228 – 240.

Hill-Burns EM, Debellius JW, Morton JT, Wissermann Wt, Lewis MR, Wallen ZD, Peddada SD, Factor SA, Molho E, Zabetian Cp, Knight R, Payami H. Parkinson's disease and PD medications have distinct signatures of the gut microbiome. Movement Disorders 2017 **32**(5): 739 – 749.

Hollister EB, Gao C, Versalovic J. Compositional and functional features of the gastrointestinal microbiome and their effect on human health. Gastroenterology 2014 **146**(6) 1449 – 1458.

Hullar MAJ, Burnett-Hartman AN, Lampe JW. Gut microbes, diet and cancer. Cancer Treatment Research 2014**159**: 377 – 399.

Humblot C, Murkovic M, Rigottier-Gois L, Bensaada M, Bouclet A, Andrieux C, Anha J, Rabot S. β-glucuronidase in human intestinal microbiota is necessary for the colonic genotoxicity of the food-borne carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline in rats. Carcinogenesis 2007 **28**(11): 2419 – 2425.

International Agency for Research on Cancer (IARC) 2018. Red meat and processed meat/ IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2015, Lyon, France) IARC monographs on the evaluation of carcinogenic risks to humans, volume 114.

Jackson MA, Goodrich JK, Maxan M-E, Freedberg DE, Abrams JA, Poole AC, Sutter JL, Welter D, Ley RE, Bell JT, Spector TD, Steves CJ. Proton pump inhibitors alter the composition of the gut microbiota Gut. 2016 May; 65(5): 749–756

Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikale M. Role of the normal gut microbiota. World Journal of Gastroenterology 2015 **21**(29) 8787 – 8803.

Javurek AB, Spollen WG, Johnson SA, Bivens NJ, Bromert KH, Givan SA, Rosenfeld CS. Effects of exposure to bisphenol A and ethinyl estradiol on the gut microbiota of parents and their offspring in a rodent model. Gut Microbes **7**(6): 471 – 485.

Joint FAO/WHO Expert Committee On Food Additives (JECFA). 2016. https://www.who.int/foodsafety/chem/jecfa/Guidance_ARfD.pdf

JECFA 2017. Summary report on the Eighty-fifth meeting (Residues of veterinary drugs) Geneva, 17–26 October 2017

104. JECFA 2018).

https://apps.who.int/iris/bitstream/handle/10665/259895/9789241210171-eng.pdf;jsessionid=D2F57DBBF206C8B3EF596864AF35E376?sequence=1

Jourova L, Anzenbacher P, Anzenbacherova E. Human gut microbiota plays a role in the metabolism of drugs. Biomedical Papers of the Medical Faculty of the University Palacky Olomouc, Czech Republic.**160**(3): 317 - 326

Jun C, Xia J, Wu S, Tu W, Pan Z, Fu Z, Wang Y, Jin Y Insights into a possible influence on gut microbiota and intestinal barrier function during chronic exposure of mice to imazalil. Toxicological Sciences 2018 **162**(1): 113 – 123.

Jun C, Zeng Z, Fu Z, Jin Y. Oral imazalil exposure induces gut microbiota dysbiosis and colonic inflammation in mice. Chemosphere 2016 **160**: 349 – 358.

Kaddurah-Daouk R, Baillie RA, Zhu H, Zeng Z-B, Wiest MM, Nguyen UT, Wojnoonski K, Watkins SM, Trupp M, Krauss RM. Enteric microbiome metabolites correlate with response to simvastatin treatment/ PLOS One 2011 **6**(10) e25482.

Kim D-H. Gut-microbiota-mediated drug-antibiotic interactions. Drug Metabolism and Deposition 2015 **43**: 1581 – 1589.

Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T, Takahashi T, Miyahuchi S, Shioi G, Inoue H, Tsujimoto G. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nature Communications 2013 doi: 10.1038/ncomms2852.

Krishnan S, Alden N, Lee K. Pathways and functions of gut microbiota metabolism impacting host physiology. Current Opinion in Biotechnology. 2015 **36**: 137–145. doi:10.1016/j.copbio.2015.08.015.

Koliada A, Syzenko G, Moseiko V, Budovska L, Puchkov K, Perederiy V, gavalko Y, Dorofeyev A, Romanenko M, Tkach S, Sineck L, Lushchak O, Vaiserman A. Association between body mass index and *Firmicutes/Bacteroidetes* ratio in an adult Ukrainian population. BMC Microbiology 2017 **17**: 120 - 125

Lai K-P, Chung Y-T, Li R, Wan H-T, Wong C K-C. Bisphenol A alters gut microbiome: comparative metagenomics analysis. Environmental Pollution 2016 **218**: 923 – 930.

Lai K-P, Ng A H-M, Wan HT Wong A Y-M, Leung C C-T,Li R, Wong C K-C Dietary exposure to the environmental chemical , PFOS on the diversity of gut microbiota, associated with the development of metabolic syndrome. Frontiers in Microbiology 2018 **9** 2552 doi10.3389/fmicb.2018.02552.

Leclercq S, Mian FM, Stanisz AM, Bindels LB, Cambier E, Ben-Amram H, Koren O, Forsythe P, Bienenstock J. Low-dose penicillin in early life induces changes in murine gut bacteria, brain cytokines and behavior Nature Communications 2017doi: 10.1038/ncomms150621.

Ley R,Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. PNAS 2005 **102**: 11070 - 11075

Li CY, Lee S, Cade S, Kuo L-J, Schulz IR, Bhatt DK, Prasan B, Bammler TK, Cui JY. Novel interactions between gut microbiome and host drug-processing genes modify the hepatic metabolism of the environmental chemicals polybrominated diphenyl ethers. Drug metabolism and Disposition 2017 **45**: 1197 – 1214

Li H, Jia W. Cometabolism of microbes and host: implications for drug metabolism and drug-induced toxicity. Clinical Pharmacology and Therapeutics 2013 **94**(5): 574 – 581.

Li H, Lin X, Zhao J, Cui L, Wang L, Gao Y, Li B, Chen C, Li Y-F. Intestinal methylation and demethylation of mercury. Bulletin of Environmental Contamination and Toxicology 2019 **102**(5):597 - 604.

Liang X, Bittinger K, Li X, Abernethy DR, Bushman FD, FitzGerald GA. Bidirectional interactions between indomethacin and the murine microbiota. eLife 2015 **4**: e08973 doi:10.7554/eLife.08973

Liang Y, Zhan J, Liu D, Luo M, Han J, Liu X, Liu G, Cheng Z, Zhou, Z, Wang P. Organophosphorus pesticide chlorpyrifos intake promotes obesity and insulin resistance through impacting gut and gut microbiota. Microbiome 2019 **7**: 19 – 33.

Licht TR and Bahl MI. Impact of the gut microbiota on chemical risk assessment. Current Opinion in Toxicology. 2015. http://doi.org.10.1016/j.cotox.2018.09.004.

Liew W-P-P, Mohd-Rezwan S, Than LTL. Gut microbiota profiling of aflatoxinB1-induced rats treated with *Lactobacillus caset* Shirota. Toxins 2019**11**:49 – 64.

Liu Q, Shao W, Zhang C, Xu C, Wang Q, Liu H, Sun H, Jiang Z, Gu A. Organochloride pesticides modulated gut microbiota and influenced bile acid metabolism in mice. Environmental Pollution 2017 **226**: 268 – 276.

Liu Y, Li Y, Liu K, Shen J. Exposing to cadmium stress cause profound toxic effect on microbiota of the mice intestinal tract. PLOS One 2014 **9**(2): e85323.

Lobach AR, Roberts A, Rowland IR. Assessing the *in vivo* data on low/ no-calorie sweeteners and the gut microbiota. Food and Chemical Toxicology 2019 **124**: 385 – 399.

Lowe P, Gyongyosi B, Satishchandran A, Iracheta-Vellve A, Ambade A, Kodys K, Catalano D, Ward DV, Szabo G. Alcohol-related changes in the intestinal

microbiome influence neutrophil infiltration, inflammation and steatosis in early alcoholic hepatitis in mice. PLOS One 2017 **12**(3):e174544.

Lowe P, Gyongyosi B, Satishchandran A, Iracheta-Vellve A, Cho Y, Ambade A, Szabo G. Reduced gut microbiome protects from alcohol-induced neuroinflammation and alters intestinal and brain inflammasome expression. Journal of Neuroinflammation 2018 **15**: 298 – 309.

Lozano VL, Defarge N, Rocque l-M, Mesnage R, Hennequin D, Cassier R, Spiroux de Vendômois L, Panoff J-M, Séralini G-E, Amiel C. Sex-dependent impact of Roundup on the rat gut microbiome. Toxicology Reports 2018 **5**: 96 – 107.

Lu L, Wan Z, Luo T, Fu Z, Jin Y. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Science of the Total Environment 2018 **631 – 632**: 449 – 458.

Mahalhal A, Williams JM, Johnson JS, Ellaby N, Duckworth CA, Burkitt MD, Liu X, Hold GL, Campbell BJ, Pritchard DM, Probert CS/ PLOS One 2018 13(10) https://doi.org/10.1371/journal.pone.0202460.

Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez KC, Dose H, Mori H, Patil KR, Bork P, Typas A. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature 2018 **555**(7698): 623 – 628.

Maier TV, Lucio M, Lee LH, VerBerkmoes NC, Brislawn CJ, Bernhardt J, Lanemdella R, McDermott JE, Bergeron N, Heinzmann SS, Morton JT, González A, Ackermann G, Knight R, Riedel K, Krauss RM, Schmitt-Kopplin P, Jansson JK. Impact of dietary resistant starch on the human gut microbiome, metaproteome and metabolome. mBio 2017 **8**(5): e01343-17

Mao Q, Manservisi F, Panzecchi S, MandrioliD, Menghetti I, Vornoli A, Bua L, Falcioni L, Lesseur C, Chen J, Belpaggi F, Hu J. The Ramazzini Institute 13-week pilot study and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome. Environmental Health 2018 **17**:50 – 61.

Mariat D, Firmesse O, Levenez F, Guimarães VD, Sokol H, DoréJ, CORTHIER g, Furet J-P. The *Firmicutes/Bacteroidetes* ratio of the human microbiota changes with age. BMC Microbiology 2009 **9** 123-128

Markowiak P, Sliżewska K. Effects of probiotics, prebiotics and symbiotics on human health. Nutrients 2017 **9**(9): 1021 – 1058.

Maurice CF, Haiser HJ, Turnbaugh PJ. .Xenobiotics shape the physiology and gene expression of the active human gut microbiome. Cell 2013 **152**(1 – 2) 39 – 50.

Meletiadis J, Turlej-Rogacka A, Lerner A, Adler, A, Tacconelli E, Mouton JW. Amplification of antimicrobial resistance in gut flora of patients treated with Ceftriaxone. Antimicrobial Agents and Chemotherapy 2017 **61**(11) e00473-17

Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, Belzer C, Palacio SD, Montes SA, Mancabelli L, Lugli GA, Rodriguez JM, Bode L, de Vos W, Gueimonde M, Margolles A, van Sindern D, Ventura M. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiology and Molecular Biology Reviews 2017 **81**(4): e00036-17.

Minalyan A, Gabrielyan L, Scott D, Jacobs J, Pisegna JR. The gastric and intestinal microbiome: role of proton pump inhibitors. Current Gastroenterology Reports 2017 **16**(8) 42 – 58.

Montassier E, Gastinne t, vangay P, Al-Ghalith GA, Bruley des Varannes SB, Massart S, Moreau P, Patel G, de La Cochetière MF, Batard E, Knights D. Chemotherapy-driven dysbiosis in the intestinal microbiome. Alimentary Pharmacology and Therapeutics 2015 **42**: 515 - 528

Mueller NT, Shin H, Pizoni A, Werlang IC, Matte U, Goldani MZ, Goldani HAS, Dominguez-Bello MG. Delivery mode and transition of pioneering gut-microbiota structure, composition and predicted metabolic function. Genes 2017 **8**: 364 – 374.

Mutlu E, Keshavarzian A, Engen P, Forsyth CB, Sikaroodi M, Gilleret P. Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. Alcoholism: Clinical and Experimental Research 2009 **33**(10): 1836 – 1846.

Nagano T, Katase M, Tsumura K. Inhibitory effects of dietary soy isoflavone and gut microbiota on contact hypersensitivity in mice. Food Chemistry 2019 **272** 33 - 38

Nasuti C, Coman MM, Olek RA, Florini D, Verdenelli MC, Cecchini C, Silvi SFedeli D, Gabbianelli R. Changes on fecal microbiota in rats exposed to permethrin during postnatal development. Environmental Science and Pollution Research 2016 **23**: 10930 – 10937.

Nguyen TLH, Vieira-Silya S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? Disease Models & Mechanisms 2015 **8:** 1 – 16.

Nielsen LN, Roager HM, casas ME, Frandsen HL, Gosewinkel U, bester K, Licht TR, Hendriksen NB, Bahl MI. Glyphosate has limited short-term effects on commensal bacterial community composition in the gut environment due to sufficient aromatic amino acid levels. Environmental Pollution 2018 **233**: 364 – 376.

Oleskin AV. Shendarov BA. Neuromodulatory effects and targets of the SCFAs and gassotransmitters produced by the human symbiotic microbiota. Microbial Ecology in Health and Disease 2016 **21**: 30971 - 30982

Patrone V, Minuti A, Lizier M, Miragoli F, Luccini F, Trevisi E, Rossi F, Callegari ML. Differential effects of coconut versus soy oil on gut microbiota composition and predicted metabolic function in adult mice. BMC Genomics 2018 **19**: 808 - 824

Petriello MC, Hoffmann JB, Vsevolozhskaya O, Morris AJ, Hennig B. Dioxin-like PCB 126 increases intestinal inflammation and disrupts gut microbiota and metabolic homeostasis. Environmental Pollution 2018 **242**: 1022 – 1032.

Pinget G, Tan J, janac B, Kaakbush NO, Angelatos AS, O'Sullivan J, Koay YC, Sierro F, Davis J, Divakarta SK, Khagal D, Moore RJ, Stanley D, Chrzanawski W, macia L. Impact of the food additive titanium dioxide (E117) on gut microbiota-host interaction. Frontiers in Nutrition 2019 **6**:57 - 69 doi:10.3389/fnut.2019.00057.

Raymond F, Ouameur AA, Déraspe M, Iqbal N, Gingras H, Dridis B, Leprohon P, Plante, P-L, Giroux R, Bérube E, Frenette J, Boudreau DK, Simard J-L, Chabot I, Domingo M-C, Trottier S, Boissinot M, Huletsky A, Roy PH, Ouellette M, Bergeron MG, Corbeil J. The initial state of the human gut microbiome determines its reshaping by antibiotics. The ISME Journal 2016 **10**: 707–720

Reygner J, Condette CJ, Bruneau A, Delanaud S, Rhazi L, Depeint F, Abdennebi-Najar L, Bach V, Mayeur C, Khorsi-Cauet H. Changes in composition and function of human intestinal microbiota exposed to chlorpyrifos in oil as assessed by the SHIME Model. International Journal of Environmental Research and Public Health 2016 **13**: 1088 – 1105.

Reygner J, Lichtenburger L, Elmhiri G, Dou S, Bahi-Jaber N, Rhazi L, deoeint F, Bach V, Khorsi-Cauet H, Abdennebi-Najar L. Inulin supplementation lowered the metabolic defects of prolonged exposure to chlorpyrifos from gestation to young adult stage in offspring rats. PLOS One 2016 **11**(10):e164614.

Ribière C, Peyret P, Parisot N, Darcha C, Déchelotte PJ, Barnich N, Peyretaillade E, Boucher D. Oral exposure to the environmental pollutant benzo[a]pyrene impacts the intestinal epithelium and induces gut microbial shifts in murine models. Nature Scientific Reports **6**: 31027 doi: 10.1038/srep31027

Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. Nature Communications 2018 **9**:3394 doi: 10.1038/s41467-018-05470-4

Roca-Saavedra P, Mendez-Vilabrille V, Miranda JM, Nebot C, Cardelle-Cobas A, Franco CM, Cepeda A. Food additives, contaminants and other minor components: effects on human gut microbiota – a review. Journal of Physiological Biochemistry 2018 **74**: 69 - 83

Rodriguez-Palacios A, Harding A, MenghiniP, Himmelmann C, Retuerto M, Nickerson KP, Lam M, Criniger CM, McLean MH, Durum SK, Pizarro TT, Ghannoum MA, Ilic S, McDonald C, Cominelli F. The artificial sweetener Splenda promotes gut *Proteobacteria* dysbiosis and myeloperoxidase reactivity in Crohn's disease-like ileitis. Inflammatory Bowel Disease **24**(5): 1005 – 1019.

Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components European Journal of Nutrition 2018 **57**:1–24

Salim SY, Kaplan GG, Madsen KL. Air pollution effects on the gut microbiota. A link between exposure and inflammatory disease. Gut Microbes **5**(2): 215 – 219.

Saad R, Rizkallah MR, Aziz RK. Gut pharmacomicrobiomics: the tip of an iceberg of complex interactions between drugs and gut-associated microbes. Gut Pathogens 2012 **4**:16 – 28.

Sahasrabudhe NM, Beukema M, Tian L, Troost B, Scholte J, Bruininx E, Bruggeman G, van den Berg M, Scheurink A, Schols HA, Faas MM, de Vos P. Dietary fiber pectin directly blocks toll-like receptor 2-1 and prevents doxorubicin-induced ileitis. Frontiers in Immunology 2018 **9**: 383 doi: 10.3389/fimmu.2018.00383

Saint-Cyr MJ, Perrin-Guyomard A, Houée P, Rolland J-G, Laurentie M. Evaluation of an oral subchronic exposure of deoxynivalenol on the composition of human gut microbiota in a model of human microbiota-associated rats/ PLOS One 2013 **8**(11): e80578.

Scheppach W, Sommer H, Kirchner T, Paganelli G-M, Bartram P, Christl S, Richter F, Dusel C, Kasper H. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 1992 **103**: 51 – 56.

Schneeberger PHH, Coulibaly JT, Guenning M, Moser W, Coburn B, Frey JE, Keiser J. Off-target effects of tribendimidine ,tribendimidine plus ivermectin, tribendimidine plus oxantel pamoate and albendazole plus oxantel pamoate on the human gut microbiota. LJP: Drugs and Drug Resistance 2018 **8**: 372 – 378.

Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body PLOS Biology 2016doi:10.1371/journal.pbio.1002533.

Shinohara K, Ohashi Y, Kawasumi K, terada A, Fujisawa T. Effect of apple intake on fecal microbiota and metabolites in humans. Anaerobe 2010 **16**(5): 510 - 515

Snedeker SM, Hay AG. Do interactions between gut ecology and environmental chemicals contribute to obesity and diabetes? Environmental Health Perspectives 2012 **120**(3): 332 - 339

Song M, Li X, Zhang X, Shi H, Vos MB, Wei X, Wang Y, Gao H, Rouchka EC, Yin X, Zhou Z, Prough RA, Cave MC, McClain CJ. Dietary copper-fructose interactions alter gut microbial activity in male rats. American Journal of Physiology: Gastrointestinal and Liver Physiology 2017 **314**(1): G119 – G130.

Staub JM, Brand L, Tran M, Kong Y, Rogers SG. Bacterial glyphosate resistance conferred by overexpression of an E. coli membrane efflux transporter. Journal of Industrial Microbiology and Biotechnology 2012 **38**(4):641 – 647.

Stedtfeld RD, Chai B, Crawford RB, Stedtfeld TM, Williams MR, Xiangwen S, Kuwahara T, Cole JR, Kaminski NE, Tiedje JM, Hashsham SA. Modulatory influence of segmented filamentous bacteria on transcriptomic response of gnotobiotic mice

exposed to TCDD. Frontiers in Microbiology 2017 **8**: 1708 doi: 10.3389/fmicb.2017.01708.

Schweirtz A, taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. Microbiota and SCFA in lean and overweight healthy subjects Obesity 2009. **18: 195 - 195**

Theilmann MC, Goh YJ, Nielsen KF, Klaenhammer, TR, Barrangou R, Hachem MA. *Lactobacillus acidophilus* metabolizes dietary plant glucosides and externalises their bioactive phytochemicals. mBio 2017 **8**(6): e01421-17.

Tu P, Gao B, Chi L, Lai Y, Bian X, Ru H, Lu K. Sub-chronic low-dose 2,4-D exposure changed plasma acylcarnitine levels and induced gut microbiome perturbations in mice. Nature Scientific Reports 2019 **9**: 4363 – 4373.

Uebanso T, Kano S, Yoshimoto A, Naito C, Shimohata T, Mawatari K, Takahashi A. Effects of consuming xylitol on gut microbiota and lipid metabolism in mice. Nutrients 2017**9**: 756 – 767.

Undark (2019) https://undark.org/2019/10/21/microbiome-scientists-debate-dysbiosis/

Unno T, Choi J-H, Hur H-G, Sadowsky MJ, Ahn Y-T, Huh C-S, Kim G-B, Cha C-J. Changes in human gut microbiota influenced by probiotic fermented milk ingestion. Journal of dairy Science 2015 **98**: 3568 - 3576

Van den Brule S, Ambroise J, Lecloux H, Levard C, Soulas R, Temmerman P-J, Palmai-Pallig M, Marbaix E, Lison D. Dietary silver nanoparticles can disturb the gut microbiota in mice. Particle and Fibre Toxicology 2016 **13**: 38 – 53.

Velmurugan G, Ramprasath T, Swaminathan K, Mithieux G, Rajendhran J, Dhivakar M, Parthasarathy A, Babu DDV, Thumburaj LJ, Freddy AJ, Dinakaran V, Puhari SSM, Rekha B, Christy YJ, Anusha S, Divya G, Suganya K, Meganathan B, Kalyanaraman N, Vasudevan V, Kamaraj R, Karthik M, Jeyakumar B, Abhishek A, Paul E, Pushpanathan M, Rajmohan RK, Velayuthan K, Lyon AR, Ramasamy S. Gut microbial degradation of organophosphate insecticides-induces glucose intolerance *via* gluconeogenesis. Genome Biology 2017 **18**: 8 – 25 doi: 10.1186/s13059-016-1134-6.Velmurugan G. Gut microbiota in toxicological risk assessment of drugs and chemicals: the need of hour. Gut Microbes 2018 **9**(5): 465 – 468.

Viennois E, Merlin D, Gewirts AT, Chassaling B. Dietary emulsifier-induced low-grade inflammation promotes colon carcinogenesis. Cancer Research 2017 **77**(1): 27 – 40.

Wang C, Yue S, Hao Z, Ren Gl, Lu D, Zhang Q, Zhao M. Pubertal exposure to the endocrine disruptor mono-2-ethylhexyl ester at body burden level caused cholesterol imbalance in mice. Environmental Pollution 2019 **244**: 657 – 666.

Wang D, Yan S, Yan J, Teng M, Meng Z, Li R, Zhou Z, Zhu W. Effects of triphenyl phosphate exposure during fetal development on obesity and metabolic dysfunctions in adult mice: impaired lipid metabolism and intestinal dysbiosis. Environmental Pollution 2019 **246** 630 - 638

Wang J, Tang L, Glenn TC, Wang J-S. Aflatoxin B_1 induced compositional changes in gut microbial communities of male F344 rats. Toxicological Sciences 2016 **150**(1): 54 – 63.

Wang L, Zhang C, Zhi X, Hou W, Li T, Pan S, Liu B, Song J, Pan Y, Ni J, Cui D. Impact of short-term exposure of AuNCs on the gut microbiota of BALB/c mice. Journal of Biomedical Nanotechnology 2019 **19** 779-789.

Wang Q-P, Browman D, Herzog H, Neely GG. Non-nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice. PLOS One 2018 https://doi.org/10.1371/journal.pone.0199080.

Wang Y, Zhang J, Wang Y, Wang K, Wei H, Shen L. Isolation and characterisation of the *Bacillus cereus* BC7 strain, which is capable of zearalenone removal and intestinal flora modification in mice. Toxicon 2018 **155**: 9 – 20.

Wang Y, Li A, Liu J, Mehmood K, Wangdui B, Shi H, Luo X, Zhang H, Li J. *L pseudomesenteroides* and *L. johnsonii* isolated from yaks in Tibet modulate gut microbiota in mice to ameliorate enteroinvasive *Escherichia coli*-induced diarrhea. Microbial Pathogenesis 2019 **133**: 1 – 9.

Werner T, Wagner SJ, Martines I, Walter J, Chang J-S, Clavel T, Kisling S, Haller D. Depletion of luminal iron alters the gut microbiota and prevents Crohn's disease-like ileitis. Gut 2010 doi 10.1136/gut.2010.216929.

Wilding LA, Bassis CM, Walacavage K, Hashway S, Leroueil PR, Morishita M, Maynard AD, Philbert MA, Bergin IL. Repeated dose (28 day) administration of silver nanoparticles of varied size and coating does not significantly alter the indigenous murine gut microbiome. Nanotoxicology 2016 **10**(5): 513 – 520.

Williams K, Milner J, Boudreau MD, Gokulan K, Cerniglia CE, Khare S. Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of Sprague-Dawley rats. Nanotoxicology 2015 **9**(3): 279 – 289.

Wortelboer K, Nieuwdorp M, Herrema H. Fecal microbiota transplantation beyond *Clostridioides difficile* infections EBioMedicine. 2019 **44**: 716–729.

Wu G, Xiao X, Feng P, Xie F, Yu Z, Yuan W, Liu P, Li X. Gut remediation: a potential approach to reducing chromium accumulation using *Lactobacillus plantarum* TW1-1. Nature Scientific Reports 2017 **7**:15000 doi 10.1038/s41598-017-15216-9.

Wu J, Wen XW, Faulk C, Boehnke K, Zhang H, Dolinoy DC, Xi C.Perinatal lead exposure alters gut microbiota composition and results in sex-specific bodyweight changes in adult mice

Wu S, Jin C, Wang Y, Fu Z, Jin Y. Exposure to the fungicide propamocarb causes gut microbiota dysbiosis and metabolic disorder in mice. Environmental Pollution 2018a. **237**: 775 – 783.

Wu S, Luo T, Wang S, Zhou J, Ni Y, Fu Z, Jin Y. Chronic exposure to the fungicide propamocarb induces bile acid metabolic disorder and increases trimethylamine in C57BL/6 mice. Science of the Total Environment 2018b **642**: 341 – 348.

Wu W, Sun M, Chen F, Cao AT, Liu H, Zhao Y, Huang X, XiaoY, Yao S, Zhao Q, Liu Z, Cong Y. Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR 43.Mucosal Immunology 2017 **10**(4): 946 - 956

Xia G, Zhong W, Zheng X, Li Q, Qiu Y, Li H, Chan H, Zhou Z, Jia W. Chronic ethanol consumption alters mammalian gastrointestinal content metabolites. Journal of Proteome Research 2013 **12**(7): 3297 – 3306.

Xu C, Lui O, Huan F, Qu J, Liu W, Gu A. Changes in gut microbiota may be early sign of liver toxicity induced by epoxiconazole in rats. Chemotherapy 2014 **60**(2): 135 – 1422.

Xia J, Jin C, Pan Z, Sun L, Fu Z, Jin Y. Chronic exposure to low concentrations of lead induces metabolic disorder and dysbiosis of the gut microbiota in mice. Science of the Total Environment 2018 **631 – 633**: 438 – 448.

Yin N, Gao R, Knowles B, Wang J, Wang P, Sun G, Cui Y. Formation of silver nanoparticles by human gut microbiota. Science of the |Total Environment 2019 **651**: 1489 – 1494.

Yoo D-H, Kim IS, Le TKV, Jung I-H, Yoo HH, Kim D-H. Gut microbiota-mediated drug interactions between lovastatin and antibiotics. Drug Metabolism and Distribution 2014 **42**: 1508 – 1513.

Zhai Q, Liu Y, Wang C, Qu D, Zhao J, Zhang H, Tian F, Chen W. *Lactobacillus plantarum* CCFM8661 modulates bile acid enterohepatic circulation and increases lead excretion in mice. Food & Function2019 **10**: 1455 – 1464.

Zhan J, Liang Y, Liu D, Ma X, Li P, Liu C, Liu X, wang P, Zhou Z. Antibiotics may increase triazine herbicide exposure risk via disturbing gut microbiota. Microbiome 2018 **6**: 224 – 236

Zhang L, Huang Y, Zhou Y, Buckley T, Wang HH. Antibiotic administration routes significantly influence the levels of antibiotic resistance in gut microbiota. Antimicrobial Agents and Chemotherapy **57**(8): 3659 – 3666.

Zhang L, Nichols RG, Correll J, Murray IA, Tanaka N, Smith PB, Hubbard TD, Sebastian A, Albert I, Hatzakis E, Gonzalez FJ, Perdew GH, Patterson AD. Persistent organic pollutants modify gut microbiota-host metabolic homeostasis in mice through aryl hydrocarbon receptor activation. Environmental Health Perspectives **123**(7): 679 - 688

Zhang P, Zhu W, Wang D, Yan J, Wang Y, Zhou Z, He L. A combined NMR- and HPLC-MS-MS based metabolomics to evaluate the metabolic perturbations and subacute toxic effects of endosulfan on mice. Environmental Science and Pollution Research 2017 **24**:18870 – 1888.

Zhang Y, Keerthisinghe TP, Han Y, Liu M, Wanjaya ER, Fang M. “cocktail” of xenobiotics at human relevant levels reshapes gut bacterial metabiome in a species-specific manner. Environmental Science and Technology 2018 **52**: 1402 – 1410.

Appendix 1

arch terms in PubMed

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

Microbiome OR microbiota AND “X” 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	Some references were found in the reference lists of papers
Fungicide	acquired in the PubMed searches.
Aldrin	
Dieldrin	
Flame retardant	
Metabolite	

Appendix 2

Abbreviations

5HT	5-hydroxytryptamine, serotonin
ABC	ATP-Binding-Cassette trans0-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
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
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	Cadmium	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 mice	Cadmium plus lead	The presence of the gut biota may have led to reduced uptake of metals in the germ-free mice.	Breton et al (2013b)

C57BL/6 mice	Lead	*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)
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* and *Enterobacteraceae* increased with vancomycin	Nagano *et al* (2019)

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 population Addition of oats or the probiotic Lactobacillus, maintained parameters at control levels	Mutlu *et al* (2009)

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	Lai *et al* (2018)

		Turicibacter, The genus *Allobaculum*, a SCFA-producing genus also increased	
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)