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Review

The Gut Microbiome at the Intersection of Xenobiotic Metabolism and Neurotoxicity

Inês Costa [1,2], Pedro Santos-Pimenta [3,4], Ana Filipa Sobral [3,5], Renata Silva [1,2] and Daniel José Barbosa [3,4,*]

[1] Associate Laboratory i4HB—Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; inessilvacosta@hotmail.com (I.C.); rsilva@ff.up.pt (R.S.)
[2] UCIBIO—Research Unit on Applied Molecular Biosciences, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] Associate Laboratory i4HB—Institute for Health and Bioeconomy, CESPU University, 4585-116 Gandra, Portugal; up202608523@edu.ff.up.pt (P.S.-P.); ana.sobral@iucs.cespu.pt (A.F.S.)
[4] UCIBIO—Research Unit on Applied Molecular Biosciences, Translational Toxicology Research Laboratory, CESPU University, 4585-116 Gandra, Portugal
[5] UCIBIO—Research Unit on Applied Molecular Biosciences, Toxicologic Pathology Research Laboratory, CESPU University, 4585-116 Gandra, Portugal
* Correspondence: daniel.barbosa@iucs.cespu.pt

Abstract

The gut microbiota is increasingly recognised as an important determinant of xenobiotic disposition and toxicity. Beyond its established roles in host physiology, the gut microbiota can directly transform xenobiotics, altering their biological activity, bioavailability, and systemic exposure. These microbial transformations may be particularly relevant to neurotoxicity, since they can modify the formation and availability of neuroactive or neurotoxic compounds that reach the central nervous system (CNS). In parallel, alterations in gut microbiota composition and microbial functional capacity can influence gut–brain communication through neural, endocrine, immune, and metabolic pathways, affecting the integrity of the intestinal and blood–brain barriers, neuroinflammation, and CNS susceptibility to toxic insults. To provide a mechanistic context for understanding how gut microbiota may modify xenobiotic-induced neurotoxicity, this review integrates these two dimensions: the microbiota-mediated xenobiotic metabolism and microbiome–brain interactions. We discuss the consequences of dysbiosis, evidence linking microbial xenobiotic transformations to neurotoxic outcomes, and the relevance of gut–brain interactions to CNS homeostasis. Emerging technologies, including functional metabolomics, microphysiological systems, and computational approaches, are highlighted as tools for establishing mechanisms and causality. Finally, we discuss translational opportunities and future challenges for incorporating microbiome function into xenobiotic risk assessment and for developing microbiome-informed strategies to predict or modify neurotoxic responses.

Keywords: xenobiotic metabolism; gut microbiota; gut–brain axis; interindividual variability; microbiome-mediated toxicity; neurotoxicity

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1. Introduction

The gut microbiota consists of a diverse community of microorganisms, primarily bacteria, but also viruses (particularly bacteriophages), archaea, fungi, and other

microbial eukaryotes. These microbial populations collectively form a complex and dynamic ecosystem that changes throughout the host's life and interacts continuously with host physiology [1], playing a fundamental role in maintaining host physiological homeostasis [2–4]. Importantly, the gut microbiota is not a static entity, since its composition and functional capacity are influenced by multiple intrinsic and extrinsic factors, including host genetics, age, diet, lifestyle, medication use, and environmental exposures [5–7]. Beyond its well-established role in digestion and nutrient absorption, the gut microbiota is now understood to contribute to the function of other organs in the host organism, including the central nervous system (CNS) [2–4,8].

Xenobiotics are generally defined as chemical compounds that are foreign to a biological system. This category encompasses a wide range of structurally and functionally diverse substances, including pharmaceutical drugs, environmental pollutants, industrial chemicals, pesticides, and dietary bioactive compounds [9]. Following exposure, xenobiotics may undergo characteristic pharmacokinetic processes, including absorption, distribution, metabolism, and excretion, which together determine their pharmacological and toxicological behaviour [10].

Traditionally, xenobiotic metabolism has been attributed primarily to host enzymatic systems, particularly hepatic enzymes such as cytochrome P450 isoenzymes and conjugation pathways [10,11]. Early studies on the gut microbiota primarily focused on nutrient metabolism and energy harvest [3], with limited consideration of its capacity to metabolise exogenous chemicals. This view has changed substantially following large-scale metagenomic studies revealing that the gut microbiome possesses a vast and functionally diverse gene repertoire, whose metabolic potential had previously been underestimated [12]. Consequently, the gut microbiota is now considered an additional metabolic hub capable of catalysing hydrolytic and reductive reactions, as well as the transfer of acetyl and methyl groups and the formation of radical intermediates, thereby modifying xenobiotic structure and activity [13,14].

In this context, the interaction between the gut microbiome and xenobiotics has important implications for toxicology, particularly in relation to neurotoxicity. The CNS is highly sensitive to chemical perturbations, and growing evidence suggests that gut microbial activity can influence neurotoxic outcomes through multiple interconnected mechanisms [15]. One key pathway involves the production of microbial metabolites that can enter the systemic circulation and potentially influence blood–brain barrier (BBB) integrity, neuroinflammatory signalling, and neuronal function [16–21]. Additionally, the gut microbiota plays a central role in regulating systemic immune responses [22], which are closely linked to neuroinflammatory processes. Dysbiosis, or disruption of the gut microbial community, has also been associated with altered immune signalling and increased vulnerability to neurotoxicity in response to chemical exposures [15,17,23]. Thus, the gut microbiome is currently viewed as an integral component of the gut–brain axis, a bidirectional communication system that links intestinal microbial activity with brain function through neural, endocrine, metabolic and immune pathways [2–4,8].

Despite growing recognition that the gut microbiome can influence neurotoxicity through both xenobiotic biotransformation and gut–brain communication, these interconnected processes remain incompletely integrated, as they are frequently examined separately [14,24,25]. As such, an integrated perspective is needed since the gut microbiome may influence neurotoxic outcomes through the direct modification of xenobiotics and their metabolites, as well as through indirect effects on biological processes that determine CNS homeostasis and susceptibility to toxic insults. Thus, understanding the role of the gut microbiome in xenobiotic metabolism and its downstream effects on the CNS is therefore essential for advancing the study of xenobiotic toxicology. Accordingly, this review explores the intersection between gut microbiota-mediated xenobiotic metabolism and

gut–brain interactions as complementary mechanisms influencing xenobiotic-induced neurotoxicity.

Several excellent reviews have addressed microbial xenobiotic biotransformation and the gut–brain axis separately. Reviews of the former have catalogued the chemistry of microbial drug metabolism [14,24,26], while reviews of the latter have described neural, endocrine, immune and metabolic communication between the intestine and the brain [2,8,27]. What has been less often attempted is the explicit connection of the two, treating microbial biotransformation as a determinant of the chemical species that reach the nervous system, and gut–brain signalling as a determinant of how vulnerable that system is when they arrive. The present review is organised around this intersection and addresses six core mechanistic themes: (i) microbial enzymes and key biochemical reactions; (ii) evidence for the biotransformation of neuroactive and neurotoxic xenobiotics; (iii) quantitative exposure, toxicokinetic considerations, and delivery across the BBB; (iv) interindividual variability driven by microbiome composition; (v) experimental models for establishing causality; and (vi) translational implications for risk assessment and therapeutics. Throughout, we prioritise transformations for which a specific enzyme, taxon, or metabolite has been identified, specify whether the evidence derives from human, animal, or in vitro studies, and distinguish between transformations demonstrated to alter neurotoxicity and those that remain mechanistically plausible but unproven.

The present review is organised around that intersection. It highlights transformations for which a specific enzyme, taxon or metabolite has been identified and distinguishes throughout between transformations that have been demonstrated to alter neurotoxicity and those that remain mechanistically plausible but unproven.

Literature Search Strategy

This is a narrative review and was not conducted, nor is it presented, as a systematic review. PubMed and Scopus were searched from database inception to August 2026, without language restriction, using combinations of the terms “gut microbiota” OR “gut microbiome” OR “intestinal microflora” AND “xenobiotic metabolism” OR “drug metabolism” OR “biotransformation” OR “azoreductases” OR “β-glucuronidase” OR “nitroreductase” AND “neurotoxicity” OR “gut-brain axis” OR “central nervous system” OR “blood-brain barrier” OR “neuroinflammation”. The reference lists of retrieved articles and of previous reviews were screened manually to identify additional sources. Records were selected by consensus among the authors.

2. The Gut Microbiome as a Metabolic Centre for Xenobiotics

In recent years, the importance of the gut microbiome in xenobiotic disposition has become increasingly evident, largely because of its ability to influence their pharmacokinetic properties. The gut microbiota can directly affect xenobiotic pharmacokinetics through two main mechanisms: by interacting with and metabolising xenobiotics through microbial metabolic pathways, and by modulating their intestinal absorption [13,28–30] (Figure 1).

Microbial metabolism encompasses a broad range of enzymatic reactions that can modify the chemical structure of xenobiotics and, consequently, their disposition and biological effects (Figure 1B). Although hydrolytic [31] and reductive [32] reactions are particularly prevalent in the gut, microorganisms can also catalyse other types of chemical transformations, including deconjugation [33,34], functional-group transfer [14,35], and C–S lyase-mediated cleavage [36], among others. The specific enzymes and mechanisms involved in these transformations are discussed in detail in Section 3.

The metabolic activity of the gut microbiota can also contribute to the presystemic metabolism of xenobiotics. A fraction of an orally administered xenobiotic may be

metabolised by gut microorganisms before reaching the systemic circulation, thereby influencing its bioavailability and systemic exposure. Thus, in addition to hepatic and intestinal first-pass metabolism, microbial metabolism in the gut can represent an additional presystemic metabolic process, contributing to the overall disposition of xenobiotics [24,37] (Figure 1B,F).

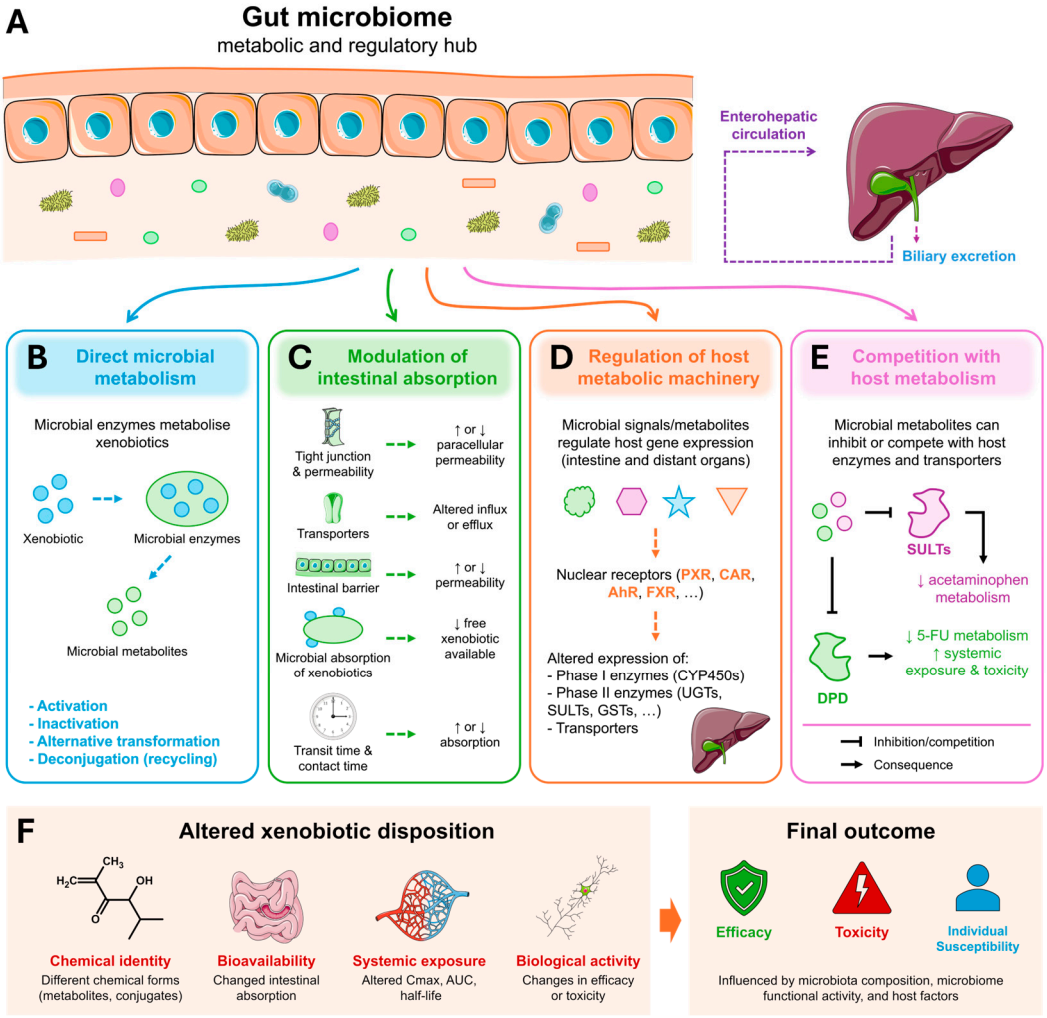

Figure 1. The gut microbiome as an integrated regulator of xenobiotic disposition and toxicological outcomes. (**A**) Following oral exposure, xenobiotics enter the gastrointestinal tract and may subsequently undergo modification by gut microbiota. Xenobiotics and their metabolites can also participate in enterohepatic cycling between the liver and the duodenum. (**B**) Direct microbial metabolism can transform xenobiotics into metabolites with altered chemical and biological properties through activation, inactivation, deconjugation, or other structural modifications. (**C**) The gut microbiota can modulate intestinal absorption by altering intestinal barrier properties, transport processes, mucus and epithelial characteristics, microbial adsorption of xenobiotics, and intestinal transit time. (**D**) Microbial metabolites and signals can regulate host xenobiotic metabolism by influencing the expression of host metabolic enzymes, transporters, and their transcriptional regulators. (**E**) Microbial metabolites can also compete with or inhibit host metabolic enzymes, thereby modifying xenobiotic metabolism. (**F**) These mechanisms, together with enterohepatic circulation, can alter xenobiotic chemical identity, bioavailability, systemic exposure, and biological activity, ultimately influencing efficacy, toxicity, and individual susceptibility. The magnitude and direction of these effects depend on microbiota composition, microbiome functional activity, xenobiotic characteristics, and host-related factors. 5-FU, 5-fluorouracil; AhR, aryl hydrocarbon receptor; AUC, area under the concentration–time curve; CAR, constitutive androstane receptor; Cmax, maximum concentration; DPD,

dihydropyrimidine dehydrogenase; FXR, farnesoid X receptor; GSTs, glutathione S-transferases; PXR, pregnane X receptor; SULTs, sulfotransferases. The illustration includes elements from Servier Medical Art. The chemical structure was created using the ChemSketch freeware [version 2023.2.4; Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada].

Microbial metabolism can also influence the enterohepatic circulation of xenobiotics (Figure 1A). Enterohepatic circulation refers to the recirculation of a substance between the liver and gut [38]. Following absorption from the gastrointestinal tract, many xenobiotics are transported through the portal circulation to the liver, where they may undergo further metabolism, particularly phase II reactions such as glucuronidation, sulphation, or glutathione conjugation. These conjugated metabolites can subsequently be excreted through the hepatobiliary system into the duodenum. Once in the gut, microbial metabolism can modify these conjugated metabolites, potentially regenerating forms of the xenobiotic that are more readily absorbed by the intestinal epithelium. Reabsorption into the systemic circulation can thereby prolong xenobiotic exposure and, in some cases, increase its apparent half-life and duration of biological effects [38].

In addition to metabolising xenobiotics, the gut microbiome can also modulate their intestinal absorption through several mechanisms (Figure 1C). These include alterations in tight junctions, transmembrane transport, intestinal barrier properties, and direct adsorption of xenobiotics [13]. Studies have shown that the gut microbiome can reduce the expression of claudin-1 and occludin at tight junctions between intestinal epithelial cells, thereby increasing paracellular permeability [39]. Conversely, other studies suggest that certain microorganisms may increase the expression of small proline-rich protein 2A, a component of the microvillar barrier of enterocytes, thereby reducing intestinal permeability [40]. Thus, the effects of the gut microbiome on xenobiotic absorption are not unidirectional and may depend on the specific xenobiotic and the microbial mechanisms involved. The presence of the gut microbiota has also been associated with reduced intestinal absorption of certain xenobiotics. Some mechanisms include shortening of intestinal microvilli and consequent reduction in the absorptive surface area [41], increased intestinal transit and reduced contact time between xenobiotics and the intestinal mucosa [42], and increased thickness and reduced permeability of the mucosal layer [39].

Comparisons between germ-free and conventional mice have shown that the absence of the gut microbiota increases passive transport across the intestinal epithelium, whereas no significant changes have been observed in active transport [43,44]. The gut microbiota can also interfere with the absorption of metals that depend on transport proteins with an affinity for cations, such as calcium transporters and metallothionein [45]. For example, the absence of gut microbiota promotes the absorption of cadmium [46], whereas the absorption of certain lipid compounds is reduced [47].

Direct adsorption of xenobiotics by microorganisms represents another mechanism through which the gut microbiota can modulate intestinal absorption (Figure 1C). Levodopa, a prodrug used in the treatment of Parkinson's disease (PD), provides an illustrative example. Following oral administration, levodopa is absorbed and transported to the CNS, where it is converted by aromatic L-amino acid decarboxylase to form dopamine. The same enzyme also acts peripherally, which is why levodopa is co-administered with a peripheral decarboxylase inhibitor [48]. However, patients infected with high levels of *Helicobacter pylori* have been reported to exhibit lower plasma concentrations of levodopa, potentially compromising its therapeutic efficacy. Subsequent studies demonstrated that *H. pylori* can bind levodopa to bacterial surface proteins, thereby reducing its availability for absorption [49,50].

Beyond these direct effects on xenobiotic metabolism and intestinal absorption, the gut microbiota can also modulate xenobiotic pharmacokinetics indirectly by altering host

gene expression (Figure 1D). This regulatory influence is not restricted to the intestine but can extend to distant organs, including the liver, thereby affecting the expression of enzymes and transporters involved in xenobiotic disposition [51].

Nuclear receptors are important regulators of the expression of genes involved in xenobiotic metabolism and transport. Their activity can therefore influence the expression of enzymes involved in phase I metabolism, such as CYP450 isoenzymes, and phase II metabolism, including uridine diphosphate glucuronosyltransferases (UGTs), sulphotransferases (SULTs), and methyltransferases, as well as transporters involved in xenobiotic efflux, including multidrug resistance proteins [45].

To investigate the influence of the gut microbiota on host gene expression, several studies have compared germ-free and conventional mice and identified substantial differences in hepatic gene expression, particularly in CYP450 isoenzymes [52–54]. One well-characterised example is CYP3A11, the murine orthologue commonly used as a functional counterpart of human CYP3A4. In germ-free mice, CYP3A11 messenger ribonucleic acid expression (mRNA), protein abundance, and enzymatic activity are significantly reduced [52–54]. This finding is particularly relevant because CYP3A4 contributes substantially to the first-pass metabolism of numerous xenobiotics, including aflatoxin B1 [55], several antibiotics [56], acetaminophen [57], and irinotecan [58]. In the intestine, many CYP3A4 substrates are also substrates of adenosine-5′-triphosphate (ATP)-binding cassette efflux transporters, particularly P-glycoprotein (P-gp), with which it shares both a broad range of substrates and common transcriptional regulators, most notably the pregnane X receptor (PXR) [59]. Efflux by P-gp returns absorbed xenobiotics to the intestinal lumen, and their repeated reabsorption prolongs exposure to enterocyte CYP3A4 while limiting intracellular accumulation. The two systems therefore act synergistically to limit oral bioavailability. Since several microbial metabolites are PXR ligands [60], microbiota-dependent changes in CYP3A4 and P-gp expression may occur in parallel, and their concomitant reduction would increase the fraction of a xenobiotic reaching the systemic circulation.

These effects are not restricted to the CYP3 family. The gut microbiota has also been shown to influence other metabolically relevant CYP450 families, including CYP1, CYP2, and CYP4, as well as non-CYP450 enzymes such as aldehyde dehydrogenases [52,61]. Similarly, microbial signals can affect enzymes involved in phase II metabolism, including glutathione S-transferases and sulphotransferases, as well as transporters responsible for xenobiotic efflux. These effects are consistent with the role of nuclear receptors and other transcriptional regulators in coordinating the expression of metabolic and transport pathways [54,61]. Overall, the extent to which these microbiota-mediated changes affect systemic xenobiotic exposure depends on the relative contribution of intestinal metabolism and efflux to the disposition of each xenobiotic.

In addition to altering host gene expression, microbial metabolites can directly modulate host metabolic pathways involved in xenobiotic disposition by inhibiting metabolic enzymes or competing with xenobiotics for enzymatic binding sites (Figure 1E). An illustrative example is the interaction between sorivudine and 5-fluorouracil (5-FU). Following oral administration, sorivudine is hydrolised to bromovinyluracil by intestinal bacteria, including members of the genus *Bacteroides* that are particularly abundant in the gut [62]. Bromovinyluracil inhibits dihydropyrimidine dehydrogenase (DPD), the principal enzyme responsible for the catabolism of the chemotherapeutic drug 5-FU [63]. The inhibition of DPD is expected to slow down the metabolism of 5-FU and, consequently, its elimination, which markedly increases systemic exposure, thereby enhancing and prolonging its toxic effects.

Enzymatic competition provides another mechanism through which microbial metabolites can alter xenobiotic disposition. One example involves the competition between p-cresol and acetaminophen. p-Cresol is generated by bacterial metabolism of tyrosine

and phenylalanine and, following absorption, can undergo hepatic sulphation to form *p*-cresol sulphate [64]. Acetaminophen also undergoes extensive hepatic phase II metabolism, primarily through glucuronidation and sulphation, generating acetaminophen glucuronide and acetaminophen sulphate, respectively [65]. Since sulphation of both p-cresol and acetaminophen is catalysed by cytosolic sulphotransferases, including SULT1A1, these compounds can compete for the same metabolic capacity [66]. This competition may reduce acetaminophen sulphation and increase the relative contribution of alternative metabolic pathways, including the pathway leading to the formation of N-acetyl-p-benzoquinone imine, a highly reactive and hepatotoxic metabolite [67].

Additionally, certain xenobiotics are capable of altering the abundance of specific gut microbial populations. Tempol, a pharmacologically active nitroxide compound with antioxidant properties [68], provides an illustrative example. Tempol alters the composition of the gut microbiota, increasing the relative abundance of Bacteroidota (formerly Bacteroidetes) and decreasing that of Bacillota (formerly Firmicutes). Within the phylum Bacillota, the genus *Lactobacillus* is particularly affected [69]. These microorganisms possess hydrolytic enzymes capable of deconjugating taurine-conjugated bile acids, thereby generating unconjugated bile acids [70]. Consequently, by depleting these bacteria, tempol administration lowers intestinal bile salt hydrolase activity and thus reduces deconjugation, consequently increasing the levels of taurine-conjugated bile acids, particularly tauro-β-muricholic acid, an antagonist of the farnesoid X receptor that is involved in the regulation of glucose, lipid, and bile acid metabolism [69,71,72].

Overall, these findings highlight the bidirectional and multifaceted nature of microbiome–xenobiotic interactions. While the gut microbiota can directly metabolise xenobiotics, alter their intestinal absorption, and indirectly influence their disposition by regulating host metabolic and transport pathways, xenobiotics can also modify the gut microbiota, potentially feeding back into host metabolism and further modifying xenobiotic disposition. Thus, the gut microbiome should be considered an integral component of xenobiotic disposition rather than merely an additional site of xenobiotic metabolism (Figure 1).

3. Microbial Enzymes and Xenobiotic Transformation

Several studies have shown that the gut microbiota carries out a variety of enzymatic reactions capable of transforming xenobiotics through chemically diverse reactions. Hydrolytic and reductive reactions are among the most extensively characterised, reflecting, in part, the low-oxygen and predominantly anaerobic environment of the intestinal lumen, which favours microbial metabolism based on electron acceptors other than molecular oxygen [26,73]. Nevertheless, microbial xenobiotic transformation also involves other enzymatic activities, including functional-group transfer, lyase-mediated cleavage, and radical chemistry. The following sections describe the major enzyme classes involved in these processes and illustrate their relevance to xenobiotic disposition and biological effects.

3.1. Hydrolases

Hydrolases are a diverse group of enzymes found both in the gut microbiota and in the host that catalyse the hydrolysis of chemical bonds in xenobiotics and their metabolites. These enzymes are found in bacteria such as *Escherichia coli*, *Enterococcus faecalis*, *Clostridium perfringens*, and *Bacteroides fragilis*, among others [26,74,75]. Hydrolytic reactions involve the cleavage of chemical bonds through the incorporation of a water molecule [14,35]. These reactions are particularly important for the gut microbiota, since the hydrolysis of complex compounds provides microorganisms with substrates that can be used for growth and energy production [31].

The main hydrolases found in the gastrointestinal tract include:

(I) Proteases, which are responsible for breaking peptide bonds, converting proteins into polypeptide chains and, subsequently, into amino acids. They can be divided into four classes, according to the sites where they carry out the bond cleavage: cysteine proteases, serine proteases, aspartic proteases, and metalloproteases [14,35]. Their distribution is not uniform throughout the gastrointestinal tract, reflecting differences in the microbial communities and physicochemical conditions along the intestine [76].

(II) Glycosidases, which are responsible for the hydrolysis of carbohydrates and glycosides. The most important glycosidases, given their widespread distribution throughout the gut microbiome, are α-rhamnosidase, β-glucosidase and β-glucuronidase [77,78]. β-Glucuronidases are particularly relevant to xenobiotic metabolism, as they hydrolyse glucuronide conjugates excreted through the hepatobiliary route, thereby reversing a major phase II metabolic reaction [79].

(III) Sulphatases, which are metalloenzymes responsible for the hydrolysis of sulphate esters generated by phase II sulphation [14,35].

(IV) Esterases, which include enzymes such as carboxylesterases, acetylesterases, and arylesterases, catalyse the conversion of ester groups into carboxylic acids and alcohols [14,35].

A particularly important consequence of microbial hydrolytic activity is deconjugation. Deconjugation reactions involve the removal of conjugated groups, such as glucuronic acid, sulphate, or glutathione-derived conjugates, that have previously been added to xenobiotics during host metabolism, particularly through phase II reactions such as glucuronidation, sulphation, and glutathione conjugation [33,34]. Many deconjugation reactions are hydrolytic and are catalysed by microbial enzymes such as β-glucuronidases and sulphatases. However, deconjugation can also occur through non-hydrolytic mechanisms, including the cleavage of cysteine metabolites derived from glutathione conjugates by microbial C–S lyases [36].

Among these hydrolases, the most significant are sulphatases and glycosidases, in particular aryl sulphatases and β-glucuronidases, respectively. These enzymes are particularly important for xenobiotic disposition because they can reverse host conjugation reactions and regenerate less polar metabolites that may be more readily absorbed by the intestinal epithelium [80].

Irinotecan is a topoisomerase I inhibitor used to treat solid tumours, including colorectal cancer [81]. This compound is metabolised by hepatic carboxylesterases to 7-ethyl-10-hydroxycamptothecin (SN-38), the active metabolite. SN-38 is subsequently conjugated with glucuronic acid in the liver via UGT, forming 7-ethyl-10-hydroxycamptothecin-glucuronide (SN-38G) [58]. Once excreted into the intestinal lumen, SN-38G can be hydrolysed by bacterial β-glucuronidases, regenerating the active metabolite SN-38 [82]. This reaction is of great importance, as SN-38 is highly toxic to enterocytes. As such, its regeneration in the gut contributes to intestinal toxicity. This mechanism has provided the rationale for the development of selective bacterial β-glucuronidase inhibitors, which can be coadministered with irinotecan to reduce SN-38G deconjugation and thereby attenuate irinotecan-induced intestinal toxicity [83].

A similar mechanism is observed following the administration of non-steroidal anti-inflammatory drugs (NSAIDs), particularly diclofenac, indomethacin, and ketoprofen [84], as well as with heterocyclic amines [85,86]. Many NSAIDs undergo hepatic phase II metabolism through glucuronidation and are subsequently excreted into the intestine through the hepatobiliary route. In the intestine, bacterial β-glucuronidases can deconjugate these metabolites, releasing the corresponding aglycones, which may subsequently be reabsorbed by intestinal epithelial cells. In the intestinal epithelium, the now-

deconjugated xenobiotic may be reconjugated, resulting in repeated exposure of the intestinal mucosa to the parent compounds. This process has been implicated in the intestinal toxicity associated with chronic NSAID use, including mucosal damage, ulceration, and, in severe cases, perforation. Additionally, the reactivation of xenobiotics can induce mitochondrial and endoplasmic reticulum stress [84,87–89].

Microbial deconjugation may also contribute to the intestinal reactivation of heterocyclic amines. These compounds, including 2-amino-3-methylimidazo[4,5-f]quinoline, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, are formed during the cooking of meat and have been associated with genotoxic effects [85,86]. They can undergo phase II metabolism in the liver to form glucuronide conjugates, which are subsequently secreted through the hepatobiliary route into the intestine. Bacterial β-glucuronidases can then deconjugate these metabolites, potentially regenerating the parent compounds and increasing their intestinal exposure [90,91].

Non-caloric artificial sweeteners (NAS), which are added to foods as sugar substitutes, may also undergo microbial transformation. Cyclamate can undergo hydrolysis of its sulphamate group by bacteria belonging to genera such as *Enterococcus*, *Clostridium*, *Corynebacterium*, *Campylobacter,* and *Escherichia*, producing cyclohexylamine [92,93], a metabolite that has been reported to exert toxic effects in animals [94]. Other low-calorie sweeteners, including xylitol [95] and saccharin [96], have also been reported to undergo microbial metabolism. Interestingly, these compounds can themselves alter the composition of the gut microbiota. In mice, chronic consumption of NAS was associated with changes in microbial composition, including increased abundance of bacteria belonging to the genus *Bacteroides* and the order Clostridiales [96]. This illustrates the bidirectional relationship between xenobiotics and the gut microbiota, in which microbial metabolism can modify xenobiotics while xenobiotics can, in turn, reshape the microbial community.

3.2. Oxidoreductases

Oxidoreductases are a group of enzymes responsible for catalysing redox reactions, involving the transfer of electrons between a donor and an acceptor. In the gut, these reactions most often correspond to the reduction of specific functional groups or chemical bonds [97]. These reactions are particularly relevant to the gut microbiome because the intestinal environment is largely anaerobic, favouring microbial metabolism based on electron acceptors other than molecular oxygen [32].

In these reductive transformations, hydrides and/or electrons are transferred to the xenobiotic from reduced cofactors such as flavin, reduced nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide phosphate, among others [14,35]. Depending on the substrate and reaction, these transformations can substantially alter the polarity, biological activity, and persistence of xenobiotics and their metabolites [98,99].

There are various types of oxidoreductases that catalyse different types of reactions. However, the most relevant are nitroreductases, which catalyse the reduction of nitro groups to amines, and azoreductases, which catalyse the reduction of azo bonds, splitting them into two amines. These enzymes are found in bacteria of the genera *Clostridium* and *Eubacterium*, such as *C. perfringens*, *Clostridium paraputrificum* and *Clostridium leptum* [100–103].

5-Aminosalicylate (5-ASA) is the active compound released from prodrugs such as sulphasalazine, olsalazine, and balsalazide, which belong to the group of aminosalicylates. To prevent its rapid absorption in the upper gastrointestinal tract and deliver the drug to the colon, 5-ASA is linked through an azo bond to a carrier moiety: sulphapyridine in sulphasalazine, a second 5-ASA molecule in olsalazine, and 4-aminobenzoyl-β-alanine in balsalazide [104,105]. For the drug to exert its pharmacological activity, the azo bond must be cleaved by bacterial azoreductases in the colon, releasing the free 5-ASA [97]. In

these compounds, bacterial azoreduction effectively functions as a colon-targeted activation step, illustrating how microbial metabolism can contribute to the site-specific delivery of a drug.

In addition to these enzymes, there are other oxidoreductases of clinical significance, such as cardenolide reductase. Digoxin is a cardiotonic glycoside whose aglycone contains an unsaturated lactone ring. This property enables it to bind to Na^+/K^+-ATPase, thereby inhibiting it [106]. Reduction of this double bond generates dihydrodigoxin, which has a lower affinity for Na^+/K^+-ATPase and consequently reduced pharmacological activity. This reduction is carried out largely by gut microorganisms, particularly *Eggerthella lenta* (formerly *Eubacterium lentum*), rather than by host enzymes [107,108]. These bacteria possess the cardiac glycoside reductase operon, which encodes enzymes capable of reducing digoxin to dihydrodigoxin. This process is of considerable clinical significance because digoxin has a narrow therapeutic window [109]. Consequently, changes in the abundance or activity of bacteria capable of metabolising digoxin may contribute to interindividual variability in its pharmacological effects.

Microbial oxidoreductases can also generate metabolites with biological activities distinct from those of the parent xenobiotics. Isoflavones, such as daidzein and genistein, belong to the class of phytoestrogens and are dietary xenobiotics commonly found in foods such as soya. These can be metabolised into equol and 5-hydroxyequol, respectively [110–112]. These metabolites, together with the secondary metabolites dihydrodaidzein and dihydrogenistein, originate exclusively from the activity of the gut microbiota. Equol, in particular, is produced by specific gut bacteria, including *Enterococcus faecium*, *Limosilactobacillus mucosae* (formerly *Lactobacillus mucosae*), and *Bifidobacterium* spp. [113,114]. These metabolites can interact with oestrogen receptors, acting as oestrogen analogues, and modulate oestrogenic signalling. Depending on the baseline oestrogen levels, they may exert either agonistic or antagonistic effects at these receptors [114]. Thus, microbial transformation can generate metabolites with biological properties that differ substantially from those of the original dietary xenobiotic.

3.3. Transferases

Transferases are a group of enzymes that catalyse the transfer of functional groups between molecules via nucleophilic substitutions. These generally require chemically activated cofactors as donors, including acetyl-coenzyme A (acetyl-CoA) and ATP. Depending on the functional group transferred, different types of reactions can occur, including methylation, acetylation, and other group-transfer reactions [14,35]. The addition or removal of functional groups can alter the chemical properties, biological activity, persistence, and bioavailability of xenobiotics. Acetylation reactions may contribute to xenobiotic detoxification in some cases. For example, 5-ASA can undergo N-acetylation in the intestinal lumen by bacterial *N*-acetyltransferases, resulting in an inactive metabolite [115]. Since bacterial azoreduction is required to release 5-ASA in the first place, the microbiota both activates and inactivates this drug, and the balance between the two activities may contribute to variability in therapeutic response.

3.4. Lyases

Lyases are a group of enzymes that catalyse the cleavage of C–C or C–X bonds (where X = O, N, S, P or halides), without involving oxidation or the addition of water [14,35]. Many xenobiotics undergo hepatic metabolism and may be conjugated with glutathione as part of phase II metabolism before being excreted through the hepatobiliary route into the intestine. Once in the intestinal lumen, microbial C–S lyases can cleave cysteine-S-conjugates derived from glutathione conjugates. For example, cysteine-S-conjugates of polychlorinated biphenyls can undergo C–S bond cleavage, generating thiol-containing

metabolites that may subsequently undergo further metabolic transformations, including methylation, promoting their bioaccumulation in adipose tissue [116]. This pathway illustrates that microbial metabolism can not only reverse host conjugation reactions but also initiate further transformations that alter the persistence and tissue distribution of xenobiotics.

Levodopa provides another example of a xenobiotic subject to microbial enzymatic transformation. In addition to the host decarboxylation described in Section 2, levodopa can also be metabolised by intestinal bacteria before absorption, through decarboxylation to dopamine [117] and subsequent p-dehydroxylation, generating m-tyramine and, in turn, m-hydroxyphenylacetic acid [118]. Bacterial strains such as *Levilactobacillus brevis* (formerly *Lactobacillus brevis*) possess tyrosine decarboxylase, which is capable of metabolising levodopa in vitro [119]. More recently, the principal interspecies pathway has been resolved. A pyridoxal phosphate-dependent tyrosine decarboxylase of *E. faecalis* decarboxylates levodopa to dopamine, and a molybdenum-dependent dopamine dehydroxylase of *E. lenta* subsequently dehydroxylates dopamine to m-tyramine. This two-organism pathway is inhibited by (S)-α-fluoromethyltyrosine, which does not affect host aromatic amino acid de-carboxylase [120]. The relative contribution of this pathway to levodopa disposition in individual patients, however, remains incompletely quantified in vivo. Together with the capacity of some microorganisms to directly bind levodopa, these findings suggest that interindividual differences in the gut microbiota may contribute to variability in levodopa exposure and therapeutic response [121].

3.5. Radical Enzymes

Radical enzymes represent another important class of microbial enzymes, particularly in anaerobic environments such as the intestinal lumen [122]. These enzymes generate high-energy radical intermediates containing an unpaired electron during catalysis. To achieve this, they utilise radicals derived from enzymes or cofactors, which can be generated through electron transfer or homolytic bond cleavage. The resulting substrate radical is subsequently converted into the product, with regeneration of the initial radical state [14]. Major classes of radical enzymes include *S*-adenosylmethionine (SAM)-dependent enzymes, cobalamin (vitamin B12)-dependent enzymes, and glycyl radical enzymes (GREs) [14].

SAM-dependent enzymes, such as chloramphenicol-florfenicol resistance proteins, and its homologue ribosomal large subunit methyltransferase N, are located in the peptidyl-transferase centre of ribosomes. By catalysing methylation reactions at the antibiotic-binding site of the ribosome, they confer resistance to several classes of antibiotics, including pleuromutilins, oxazolidinones, and lincosamides [123]. This example illustrates how microbial enzymatic activity can indirectly influence xenobiotic exposure by altering the composition of the gut microbiota and its functional capacity [124,125]. Certain GRE-dependent pathways can result in the formation of *p*-cresol, which may compete with xenobiotics for host metabolic enzymes, particularly those involved in O-sulphation, thereby potentially impairing the detoxification of certain xenobiotics [126].

Glycine radical enzymes, found in bacteria belonging to the phyla Bacillota, Proteobacteria, and Actinobacteria, also participate in the metabolism of dietary and endogenous compounds. Although not representing xenobiotic transformation in the strict sense, these pathways illustrate how microbial enzymatic activity can generate metabolites with systemic biological effects. One well-characterised example is the GRE-catalysed conversion of choline-containing compounds into trimethylamine (TMA) [127]. TMA can be absorbed and transported to the liver, where it is oxidised by flavin-containing monooxygenases to form trimethylamine N-oxide (TMAO) [128]. TMAO has been associated with cholesterol accumulation in macrophages and foam-cell formation [129], as well as increased risk of

cardiovascular disease [130]. A similar microbial pathway occurs with L-carnitine, which is abundant in red meat and can also be converted into TMA, although through distinct enzymatic routes, linking microbial metabolism of dietary compounds to pathways implicated in atherosclerosis [131].

Overall, microbial enzymes can transform xenobiotics through a chemically diverse range of reactions, including hydrolysis, reduction, functional-group transfer, lyase-mediated cleavage, and radical chemistry. Deconjugation represents an important functional consequence of several of these reactions, particularly hydrolysis and C–S lyase-mediated cleavage. These transformations can detoxify xenobiotics, activate prodrugs, regenerate previously conjugated compounds, or generate metabolites with distinct biological activities. Consequently, the functional capacity of the gut microbiome represents an important determinant of the chemical and biological consequences of xenobiotics in the gastrointestinal tract.

4. Consequences of Gut Microbiota-Mediated Xenobiotic Transformation

Microbial transformation of xenobiotics can have important pharmacological and toxicological consequences, depending on whether it alters the activity, bioavailability, persistence, or toxicity of the parent compound or generates metabolites with distinct biological properties [26,30]. These effects can range from microbial activation of prodrugs and modulation of therapeutic efficacy to drug inactivation, regeneration of biologically active compounds following host metabolism, and the formation of toxic or reactive metabolites. Importantly, such changes may extend beyond the gastrointestinal tract, as microbial metabolism can alter the systemic exposure to xenobiotics and their metabolites and thereby influence their effects on distant organs, including the CNS [24,37,51].

A significant consequence of gut microbial metabolism is the activation of prodrugs, in which microbial enzymes convert pharmacologically inactive or less active compounds into metabolites with biological activity (Figure 2). In some cases, this microbial bioactivation can also be crucial for the therapeutic success of certain drugs. Sulphasalazine, an anti-inflammatory drug used in the treatment of inflammatory bowel disease and rheumatoid arthritis, provides a well-characterised example. This drug consists of a conjugate of sulphapyridine and 5-ASA linked by an azo bond. In this form, it is largely resistant to human digestive enzymes and poorly absorbed in the upper gastrointestinal tract. However, upon reaching the colon, bacterial azoreductases cleave the azo bond, releasing 5-ASA, which exerts local anti-inflammatory effects, while sulphapyridine is subsequently absorbed systemically. This microbial activation ensures the targeted delivery of the active metabolite to the site of inflammation in the colon, maximizing therapeutic effects and minimizing systemic side effects. This mechanism was established in rodents and subsequently confirmed in patients, in a controlled trial that identified 5-ASA as the active therapeutic moiety [132,133]. More recent studies also demonstrated that sulphasalazine modulates the gut microbiota composition. It has been associated with enhanced abundance of bacteria known for their beneficial potential, such as *Faecalibacterium prausnitzii*, and with increased butyrate production, while reducing certain potentially pathogenic bacterial populations, which may contribute to its therapeutic effects [134–137].

Prontosil is another example of a microbially activated prodrug, being converted by gut microbial azoreductases into its active form, sulphanilamide, which exerts the antibacterial effect, as shown in the rat [138,139]. The same principle has been applied in the design of colon-targeted anticancer prodrugs: azo-conjugated derivatives of agents such as methotrexate and gemcitabine remain stable at gastric and intestinal pH and are cleaved by microbial azoreductases, releasing the parent drug in the colon in ex vivo models [140]. These examples illustrate that the gut microbiota can function not only as a

determinant of xenobiotic metabolism but also as an active biological component of drug activation.

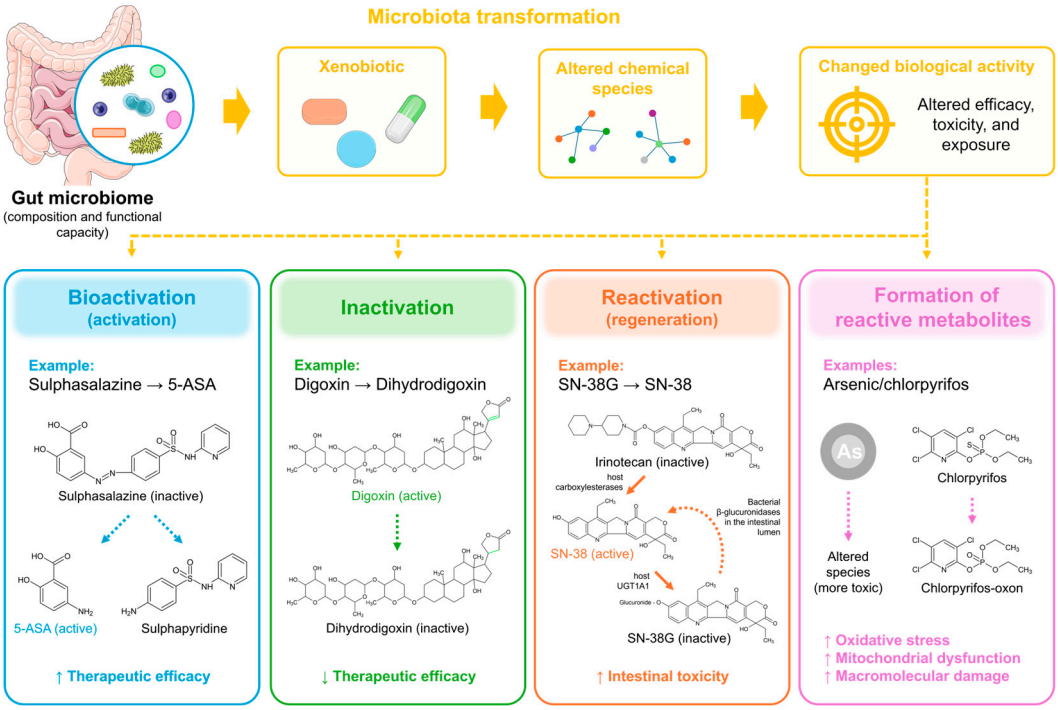

Figure 2. Gut microbiome-mediated transformation of xenobiotics and its potential impact on biological activity. The gut microbiome can transform xenobiotics into chemically altered species, thereby modulating their biological activity, efficacy, toxicity, and systemic exposure. Microbial metabolism may result in bioactivation, generating more pharmacologically active compounds; inactivation, reducing the biological activity of the parent compound; reactivation (regeneration), restoring the activity of previously inactivated metabolites; or the formation of more reactive metabolites, which may promote oxidative stress, mitochondrial dysfunction, and macromolecular damage. 5-ASA, 5-aminosalicylic acid; SN-38, 7-ethyl-10-hydroxycamptothecin (irinotecan metabolite); SN-38G, SN-38 glucuronide; UGT1A1, UDP-glucuronosyltransferase 1A1. The illustration includes elements from Servier Medical Art. Chemical structures were created using the ChemSketch freeware [version 2023.2.4; ACD/Labs, Toronto, ON, Canada].

Conversely, microbial metabolism can reduce the efficacy of active xenobiotics by converting them into less active or inactive metabolites, potentially reducing systemic exposure to therapeutically effective concentrations (Figure 2). Digoxin, a cardiac glycoside used to treat heart failure and atrial fibrillation, provides a well-established example. Certain gut bacteria, particularly *E. lenta*, express enzymes capable of reducing digoxin to its inactive metabolite, dihydrodigoxin, diminishing its therapeutic effect. Variation in the abundance and metabolic activity of digoxin-reducing bacteria may therefore contribute to interindividual differences in drug response. This was demonstrated using human faecal samples together with gnotobiotic mice colonised with *E. lenta* [99,141].

Another example is the microbial decarboxylation of levodopa to dopamine in the intestinal lumen [117,120] (see Section 3; a conversion first documented in the human digestive tract and subsequently re-constituted in vitro and in mice), which can reduce levodopa availability for systemic absorption, further illustrating how microbial metabolism may alter host exposure to pharmacologically active compounds.

Microbial metabolism can also increase toxicity through the regeneration of compounds that have previously undergone host detoxification (Figure 2). Irinotecan illustrates this mechanism. Following SN-38 regeneration in the intestinal lumen by bacterial

β-glucuronidases, the increased concentrations of SN-38 in the intestinal lumen cause severe diarrhoea in patients, a major side effect of irinotecan treatment, which can limit the treatment dosage and efficacy [142]. This intestinal injury can also compromise epithelial barrier integrity, potentially increasing intestinal permeability and the systemic absorption of other xenobiotics and their metabolites.

The biotransformation of xenobiotics by the gut microbiota can also convert them into metabolites that are more toxic than the parent compound, thereby increasing the risk of toxicity (Figure 2). Arsenic represents a relevant example. Gut microorganisms can transform inorganic arsenic through reduction and methylation, as shown in in vitro models of the human colon, thereby altering arsenic speciation and generating chemical species with distinct toxicological properties, since trivalent methylated arsenicals are considerably more cytotoxic and genotoxic than their inorganic precursors, whereas the corresponding pentavalent species are less [143,144]. Another example is the organophosphate pesticide chlorpyrifos, which intestinal bacteria degrade in vitro through species-dependent pathways: *Lactobacillus fermentum* converts it mainly to 3,5,6-trichloro-2-pyridinol, whereas *Lactococcus lactis* and *E. coli* yield chlorpyrifos-oxon, a substantially more potent inhibitor of acetylcholinesterase than the parent compound [145,146]. Microbial degradation of chlorpyrifos cannot therefore be equated with detoxification, since the metabolite formed depends on which species predominate. These findings provide mechanistic examples in which the microbial community can modify the toxic potential of environmental xenobiotics by altering their chemical identity and the amount of parent compound available for host metabolism.

Additionally, microbial metabolism can generate reactive intermediates that contribute to off-target effects and may exacerbate drug toxicity [24,147]. For instance, host metabolism of cyclophosphamide, an anticancer and immunosuppressive drug, produces the highly reactive metabolite acrolein, which is filtered in the kidneys and concentrates in the bladder. Acrolein induces the production of reactive oxygen species and nitric oxide, leading to severe bladder toxicity in rats and in treated patients [148,149]. In addition to its well-established role in urotoxicity, the high chemical reactivity of acrolein allows it to exert toxic effects in multiple tissues [150,151]. Although the conversion of cyclophosphamide to acrolein does not occur through gut microbial metabolism, the gut microbiome can independently contribute to acrolein formation through distinct metabolic pathways. For instance, microbial metabolism of glycerol can generate acrolein, identifying the gut microbiome as a potential endogenous source of this highly reactive and toxic aldehyde. These findings relied on bacterial culture and on the detection of the responsible genes in faecal metagenomes, and luminal acrolein concentrations have not been measured in vivo [152]. Consequently, differences in microbial metabolic capacity may influence local and potentially systemic exposure to acrolein, thereby modulating susceptibility to its toxic effects.

Similarly, microbial metabolism of azo and nitro compounds, found in certain drugs (e.g., nitrazepam, for which the microbial contribution to teratogenicity was shown in rats) and environmental pollutants (e.g., 2,4,6-trinitrotoluene), generates reactive arylamine intermediates, which may form adducts with host proteins or deoxyribonucleic acid, potentially leading to genotoxicity or immune activation [24,147,153,154]. Additionally, xenobiotics such as heterocyclic amines, formed during high-temperature cooking of meat, are also metabolised by gut microbial β-glucuronidases into reactive intermediates, enhancing their genotoxicity, as demonstrated in gnotobiotic rats. The corresponding association with colon cancer risk in humans remains epidemiological [91,155].

Overall, these examples illustrate that gut microbiome-mediated xenobiotic transformation can have diverse pharmacological and toxicological consequences through multiple, mechanistically distinct pathways. Microbial metabolism may activate or inactivate

xenobiotics, increase the formation of toxic metabolites, alter the chemical species available for systemic absorption, regenerate biologically active compounds following host detoxification, or contribute to the formation of reactive intermediates. Consequently, the biological and toxicological activity of a xenobiotic cannot necessarily be inferred solely from the parent compound, as microbial metabolism can modify the balance between the parent compound and metabolites with distinct chemical and biological properties [26,30].

Additionally, interindividual variation in the gut microbiota composition and microbiome functional capacity may contribute to differences in xenobiotic response by influencing which microbial transformations occur and, consequently, the formation, absorption, and systemic exposure of biologically active or toxic metabolites. Thus, microbiome-dependent differences in xenobiotic metabolism may represent an important source of interindividual variability in susceptibility to xenobiotic-induced toxicity and other biological effects [30,156].

5. Variability in Xenobiotic Metabolism Due to Microbiota Diversity

The gastrointestinal tract harbours a highly diverse microbial community whose composition varies substantially among individuals. Although an individual may harbour several hundred microbial species, thousands of microbial taxa are represented collectively across human populations. Despite this taxonomic variation, the abundance of many genes involved in basic or housekeeping metabolic functions may be relatively conserved between individuals [157,158]. However, functional redundancy in core microbial processes does not necessarily imply equivalent capacity for xenobiotic metabolism. The abundance, distribution, expression, and activity of microbial genes and enzymes involved in the transformation of xenobiotics can vary substantially between individuals [30]. Consequently, differences in gut microbiota composition and functional capacity may result in interindividual variation in the extent and nature of xenobiotic biotransformation, influencing the metabolites formed and their subsequent absorption, systemic exposure, and biological effects [30].

Several factors influence gut microbiota composition and its functional capacity, including diet, age, host genetics, lifestyle, medication use, and environmental exposures. By modifying the microbial ecosystem and its metabolic repertoire, these factors may in turn influence the capacity of the gut microbiome to transform xenobiotics and, consequently, their toxicological and other biological effects.

5.1. Diet

Food is the primary source of energy for gut microorganisms, and changes in host dietary patterns may account for up to 57% of rapid changes in gut microbiota composition [159]. Long-term dietary habits also influence the composition and metabolic activity of microorganisms residing in the human gut [160]. Likewise, short-term consumption of diets composed exclusively of plant- or animal-based products can significantly alter microbial community composition and accentuate interindividual differences in microbial gene expression [161].

Carbohydrates are the primary source of carbon and energy for colonic microorganisms. Dietary fibres are microbiota-accessible carbohydrates mainly found in cereals, fruits, and vegetables. They are fermented by colonic bacteria through saccharolytic processes, yielding monosaccharides, short-chain fatty acids (SCFAs), including butyrate, acetate, and propionate, as well as gases such as carbon dioxide and methane [162]. Additionally, dietary fibres promote the growth of microorganisms such as *Lactobacillus* and *F. prausnitzii*, which are associated with anti-inflammatory effects and SCFA production, thereby contributing to a more stable, resilient, and anti-inflammatory gut ecosystem.

Collectively, these effects may contribute to improved gut barrier function [163]. By contrast, a low-carbohydrate diet may increase *Bacteroides thetaiotaomicron* abundance in faecal samples, thereby promoting the degradation of intestinal mucus glycans and thinning of the intestinal mucus barrier [164].

Proteins are linear chains of amino acids linked by peptide bonds. Through proteolytic fermentation, which occurs predominantly in the distal colon, proteins can generate a variety of metabolites, including SCFAs, although generally in lower quantities than during saccharolytic fermentation, branched-chain fatty acids (BCFAs), such as isobutyrate, 2-methylbutyrate, and isovalerate, and potentially harmful metabolites, including ammonia, nitrosamines, and TMA [163,165].

A diet rich in animal-derived proteins, particularly red meat and dairy products, may favour bile-tolerant anaerobic bacteria such as *Bacteroides*, *Alistipes*, and *Bilophila*. The high concentrations of choline and L-carnitine found in red meat can be converted to TMA by members of the gut microbiota, including certain *Candida*, *Campylobacter*, and *Shigella* species and *Ruminococcus gnavus*. TMA is subsequently absorbed into the portal circulation and oxidised to TMAO by host hepatic enzymes [130]. TMAO may reduce bile acid synthesis and reverse cholesterol transport, thereby decreasing intestinal cholesterol elimination [166]. High consumption of animal-derived proteins may also promote the growth of sulphate-reducing bacteria, such as *Desulfovibrio* spp., which produce hydrogen sulphide from dietary inorganic sulphur and sulphur-containing amino acids, potentially contributing to gut inflammation [167].

The intake of plant-derived proteins may increase the abundance of potentially beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, while decreasing the abundance of *B. fragilis* and *C. perfringens* [168]. Plant-derived proteins may therefore represent an alternative to animal proteins with the potential to contribute to the reduction in gut inflammation.

Dietary protein is a major source of nitrogen for colonic microbial growth and is essential for carbohydrate assimilation and the production of beneficial metabolites, such as SCFAs. Therefore, the simultaneous availability of protein and carbohydrates in the large intestine may synergistically promote microbial metabolic activity and gut health [169].

Dietary fat also influences the gut microbiota and metabolic health. A high-fat diet can lead to decreases in Bacteroidota and increases in Bacillota and Pseudomonadota (formerly Proteobacteria), potentially promoting increased gut permeability, adipose tissue inflammation, and insulin resistance [170]. It may also result in increased circulating levels of bacteria-derived lipopolysaccharide (LPS) [171]. Monounsaturated fatty acids have been positively associated with the *Parabacteroides*, *Prevotella*, and *Turicibacter* genera and the *Enterobacteriaceae* family, and may exert beneficial health effects through their association with increased gut microbiota diversity [172]. Medium-chain fatty acids may increase the abundance of *Bifidobacterium* and *Lactobacillus* and have been associated with improvements in metabolic and cognitive functions, weight loss, and lipid catabolism, possibly by refining gut barrier integrity and microbial equilibrium [173]. Polyunsaturated fatty acids (PUFAs), including omega-3 PUFAs, may restore the Bacillota/Bacteroidota ratio and increase butyrate-producing taxa within the *Lachnospiraceae* family. However, increased omega-6 PUFA intake combined with reduced omega-3 PUFA intake may exacerbate dysbiosis, contributing to metabolic disorders and alterations in gut barrier function [174].

Different dietary patterns may also influence the gut microbiota. The Western diet, characterised by high consumption of highly refined foods, sugars, saturated fats, animal-derived proteins, and processed foods, is associated with a high incidence of chronic diseases, including cardiovascular disease, type 2 diabetes, and colorectal cancer [169]. Long-term consumption of this dietary pattern can also affect the gut microbiota. It has been

associated with increased abundances of potentially pathogenic microorganisms, including *certain* Bacillota, Pseudomonadota, *Alistipes* spp., *Bilophila* spp., and members of the *Enterobacteriaceae* family, and with reduced abundances of microorganisms generally considered beneficial, including *Lactobacillus* spp., *Roseburia* spp., *Agathobacter rectalis* (formerly *Eubacterium rectale*), *Bifidobacterium* spp., and *Enterococcus* spp. [175].

The Mediterranean diet, characterised by regular consumption of fibre-rich foods, nuts, and olive oil, has been associated with a reduced Bacillota/Bacteroidota ratio and lower *Escherichia coli* abundance, while increasing gut microbiota diversity, including higher levels of *Bifidobacterium*, *Lactobacillus*, *Oscillospira*, *Faecalibacterium*, *Roseburia*, and *Clostridium* cluster XIVa. This dietary pattern also favours SCFA production, potentially contributing to disease prevention [176,177].

Finally, a vegetarian diet has been associated with alterations in the *Bacteroides*/*Prevotella* ratio and increased abundances of *B. thetaiotaomicron*, *Clostridium clostridioforme*, *Klebsiella pneumoniae*, and *F. prausnitzii*, together with lower levels of *Bilophila wadsworthia* [178,179]. These changes may result in decreased levels of potentially harmful metabolites, such as lithocholic acid and aromatic compounds, and increased SCFA production [180].

Thus, dietary patterns can influence not only the composition of the gut microbiota but also its functional capacity, potentially affecting the diverse biological and metabolic processes mediated by the microbiota and contributing to interindividual variability in gut microbiota-dependent functions.

5.2. Age

Some evidence indicates that age is associated with changes in gut microbiota composition and diversity. The development of the human microbiome may begin before birth. Although microbial signals have been reported in the placenta, amniotic cavity, umbilical cord, and meconium, the existence of a stable in utero microbiome remains debated [181–184]. The composition of the gut microbiota can be influenced by several factors, including the mode of delivery and feeding, maternal diet and microbiota composition, antibiotic exposure, and the surrounding environment. Vaginal delivery promotes early and enriched colonisation by *Bacteroides*, lactobacilli, and *Prevotella*. In contrast, infants born by caesarean section show lower colonisation by *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* and are more frequently colonised by *Clostridioides difficile*, *C. perfringens*, and *E. coli* [181,185–187].

Feeding mode also influences gut microbiota composition. Breastfed infants generally show higher abundances of *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, and *Staphylococcus*, whereas formula-fed infants may exhibit higher abundances of *Bacteroides*, clostridia, and *Pseudomonadota* [188,189]. The next major transition in microbiota composition occurs with the introduction of solid foods, after which the microbiota continues to develop until approximately three years of age, when it acquires a more stable, adult-like structure [189]. An unhealthy and poorly varied maternal diet, as well as antibiotic exposure, can also affect the microbiota of children, reducing microbial diversity while potentially promoting the expansion of pathogenic or opportunistic microorganisms. Additionally, antibiotic exposure during the first two years of life has been associated with multiple diseases, including atopic dermatitis, allergic and inflammatory diseases, obesity, and attention-deficit/hyperactivity disorder [190]. Thereafter, between 2 and 5 years of age, the composition of the gut microbiota becomes increasingly stable, with the establishment of communities predominantly comprising members of the Bacillota and Bacteroidota phyla [191].

The adult gut microbiota is generally highly diverse and relatively stable, with the consistent presence of taxa such as *Faecalibacterium*, *Bacteroides*, and *Ruminococcus*.

Nonetheless, diet, lifestyle, medication use, geographical location, and socioeconomic status can all influence its composition [192]. In older adults, microbial diversity tends to decline, potentially as a consequence of inflammageing, immune dysfunction, and malnutrition. An increase in *Pseudomonadota*, which is often associated with inflammation, and a decrease in *Bifidobacterium* and *F. prausnitzii* have been reported [193]. Additionally, SCFA production may decrease with ageing, potentially reflecting lower fibre intake and the cumulative effects of antibiotic treatment [194].

While age itself plays an important role in shaping the gut microbiota, environmental factors acting throughout an individual's life also exert a significant influence on its composition and function. Therefore, age-related changes in gut microbiota composition and microbiome function may contribute to differences in microbiota-mediated biological processes throughout life.

5.3. Genetics

Host genetics also influence the gut microbiota by modulating the environment in which microorganisms colonise and persist. Specific host genes may influence microbial colonisation by regulating gut pH, immune tolerance, nutrient availability, and mucin production. Studies in twins have shown that some microbial taxa are more heritable than others. For example, members of the Christensenellaceae family and *Bifidobacterium* have been found to be influenced by host genetics, showing higher concordance in identical twins [195]. Another key example is the *LCT* gene, which encodes lactase, the brush-border enzyme responsible for lactose digestion. In individuals with persistent lactase expression, a phenotype known as lactase persistence, lactose is hydrolysed in the small intestine and little reaches the colon. In contrast, in lactase-nonpersistent individuals, undigested lactose reaches the colon, where it becomes available to lactose-fermenting bacteria; accordingly, the *LCT* genotype has been associated with differences in the abundance of taxa such as *Bifidobacterium* [196].

Thus, host genetics, by influencing gut microbiota composition and diversity, as well as microbiome function [197], may also contribute to interindividual differences in its composition and functional capacity, potentially influencing microbiota-mediated processes.

5.4. Lifestyle and Environmental Exposures

Lifestyle factors play an important role in shaping the gut microbiota, influencing its diversity, composition, and metabolic activity. For example, exercise is an important modulator of the gut microbiota and has been associated with increased abundances of potentially beneficial microorganisms, such as *Akkermansia*, *Bacteroides*, and *Faecalibacterium*, improved gut barrier function, and reduced inflammation. In addition, exercise appears to increase the production of SCFAs, particularly butyrate, and reduce circulating markers of inflammation [198,199]. Some studies have reported that professional athletes exhibit an increased abundance of metabolic pathways associated with SCFA production, further highlighting the potential beneficial effects of physical activity on the gut microbiome [200].

Circadian rhythms also play an important role in regulating the gut microbiome, with the two systems forming a bidirectional relationship. Briefly, a synchronised circadian rhythm supports a balanced gut microbiome, whereas circadian disruption can lead to microbial imbalance. Several studies have demonstrated that the intestinal microbiota both participates in and regulates circadian rhythms, potentially contributing to diseases associated with circadian rhythm disruption [201]. Likewise, sleep also influences the gut microbiome. Sleep disorders, including insomnia and irregular sleep patterns, have been associated with a 12% higher risk of colorectal cancer. In addition, individuals with short

sleep duration have been reported to exhibit lower levels of human defensin 5, an antimicrobial peptide secreted by Paneth cells in the small intestine that targets pathogenic bacteria and contributes to the regulation of gut microbiota composition [202]. Reduced defensin 5 may impair gut microbial defences, potentially increasing intestinal permeability and contributing to gut microbiota dysbiosis [203].

Stress also influences the gut microbiota and the normal functioning of the gastrointestinal tract. Some studies have reported that chronic stress reduces the abundance of *Lactobacillus* in the gut, particularly *Lactobacillus johnsonii*, which may play an important role in maintaining immune function through its interactions with leukocytes, thereby helping to counteract infections and inflammation [204]. In addition, stress can alter the abundance of specific bacterial taxa, increasing *Escherichia* and *Shigella* levels while reducing potentially beneficial bacteria, such as *Eubacterium* and *Roseburia* [205]. Stress can also increase intestinal permeability, thereby promoting inflammation, and interfere with mucin secretion and gut motility, collectively contributing to disruption of the microbial balance [206].

The environment in which individuals live also influences gut microbiota composition. This factor is interconnected with diet, urbanisation, exposure to pollutants, and other lifestyle-related factors. For example, individuals living in rural or natural environments appear to have greater gut microbiota diversity than those living in urban areas [207]. Similarly, exposure to air pollutants, which is generally more common in urban environments, has been associated with reduced microbiota diversity [208].

Collectively, lifestyle and environmental factors can modify the gut microbiota composition and its functional capacity, potentially influencing the diverse biological processes mediated by the microbiota.

Overall, gut microbiota composition and its functional capacity are shaped by multiple interacting factors, including diet, age, host genetics, lifestyle, and environmental exposures. Consequently, substantial differences may exist between individuals in both the microorganisms present in the gut and the biological functions they perform. Moreover, because several of these factors change throughout life, the gut microbiome is a dynamic ecosystem whose composition and functional capacity may also vary within the same individual over time. This variability may influence multiple microbiota-mediated processes, including xenobiotic transformation, and consequently contribute to differences in the biological effects of xenobiotic exposure.

Variability of a second, largely independent kind should be considered alongside this. The factors discussed above determine how much of a given metabolite can be formed in an individual, but not how far that signal can be transmitted once formed [30,156,209]. Transmission depends on host properties that themselves vary substantially between people. The density and sensitivity of vagal afferents and the receptors they express [210–212], baseline intestinal permeability and the integrity of the epithelial barrier [213,214], the responsiveness of enteroendocrine cells [215], the reactivity of the mucosal and systemic immune compartments [216], and the integrity and transporter complement of the BBB [217–219], where expression of P-gp and breast cancer resistance protein differs between individuals and is further altered by disease and by concomitant medication. Two individuals whose microbiota generate the same metabolite in the same amount may therefore differ considerably in the central consequence, and two individuals with identical gut–brain signalling may differ because only one of them carries the microbial capacity to form the metabolite at all. Since the two axes are shaped by largely different determinants, it is their joint distribution rather than either alone that governs the spread of neurotoxic outcomes in a population. This has a practical implication for the predictive strategies discussed in Sections 9 and 11. Microbiome profiling, however refined, characterises only one of the two axes, and will therefore systematically underestimate interindividual

variability in neurotoxic response unless host-side measures of barrier function, immune reactivity and central delivery are measured alongside it.

6. Gut Dysbiosis and Altered Xenobiotic Metabolism

Gut dysbiosis refers to a disturbance in the gut microbiota composition and diversity and/or microbiome functional capacity that alters host–microbiome interactions. Rather than representing a single, uniform microbial state, dysbiosis may involve losses or expansions of specific microbial populations, changes in community structure, and alterations in microbial metabolic functions. Depending on the biological context, these alterations may include reductions in populations commonly associated with beneficial functions, including *Bifidobacterium*, *Lactobacillus*, and *F. prausnitzii*, together with the expansion of potentially harmful microorganisms such as *C. difficile*. Several factors contribute to this state, including a poor diet, the use of antibiotics or other medication, stress, and some disease states [220,221].

Since the term is used across a heterogeneous literature, it is worth stating how it is used here and how it was defined in the studies cited. We use "dysbiosis" only as shorthand for a measured deviation from a stated reference population, and not as a diagnosis or as a uniform biological state. The studies discussed below define that deviation in different ways: as reduced α-diversity, as an altered ratio between the Bacillota and Bacteroidota phyla, as the loss or expansion of particular taxa, or as a change in functional gene content [220,221]. These definitions are not interchangeable, and two communities classified as dysbiotic by one criterion may be indistinguishable by another. For xenobiotic metabolism, the distinction matters, because what determines whether a transformation occurs is not compositional distance from a reference but the presence and expression of the responsible enzymes, principally azoreductases, nitroreductases, β-glucuronidases, sulphatases and amino acid decarboxylases [14,24]. Two taxonomically distinct communities may therefore be functionally equivalent for a given reaction, while two similar communities may differ substantially if the relevant gene is carried or expressed by only one of them [209,222]. Throughout this section, where a cited study characterised the microbiota taxonomically without measuring the corresponding functional capacity, this is stated explicitly.

As discussed in the previous section, several environmental and host-related factors can influence gut microbiota composition and microbiome functional capacity. Persistent or substantial disturbances in these factors may promote dysbiosis, thereby altering not only the structure of the microbial community but also the functions mediated by the gut microbiota. Diet represents an important contributor, as changes in food composition, as well as food shortage or oversupply, can alter the gut microbial ecosystem and promote dysbiosis and associated diseases [223].

Antibiotic exposure is another major cause of dysbiosis. Although antibiotics are designed to target disease-causing bacteria, their limited specificity can also affect commensal microorganisms, leading to reduced microbial diversity, alterations in the abundance of specific taxa, increased abundance of Pseudomonadota, including *Enterobacteriaceae*, and changes in the expression of antibiotic resistance genes [224]. Antibiotic-induced alterations in the gut microbiota may also affect intestinal barrier integrity and immune function, increasing susceptibility to intestinal disorders [225,226]. The magnitude and persistence of these effects depend on several factors, including antibiotic class, mode of action, route of administration, and treatment duration, as well as host characteristics. For example, a study comparing metronidazole and oral vancomycin in mice showed that vancomycin caused more pronounced disruption of the gut microbiota [227].

Certain populations, including neonates and children, may be particularly vulnerable to antibiotic-induced alterations in the gut microbiota. For instance, the use of

antibiotics in childhood can promote a decrease in the abundance and diversity of microbial species, especially Bifidobacterium [228]. Antibiotic exposure is also associated with antibiotic-associated diarrhoea and *C. difficile* infection, and the multi-antibiotic regimens used for *Helicobacter pylori* eradication have themselves been shown to perturb the gut microbiota [229].

Importantly, although some antibiotic-induced changes may recover over time, disturbances in microbial composition and function can persist for weeks to months and, in some cases, for as long as two years after exposure. For example, alterations in the microbial composition of healthy adult volunteers were still detected two years after a one-week use of clindamycin [230].

Since dysbiosis alters gut microbiota composition and the functional repertoire of the gut microbiome, it can modify microbiota-mediated xenobiotic transformation. Changes in the abundance of microorganisms capable of performing specific metabolic reactions may reduce or enhance the formation of particular xenobiotic metabolites, while alterations in microbial community structure may favour alternative metabolic pathways. Consequently, dysbiosis may influence the activation or inactivation of xenobiotics, the formation of toxic or biologically active metabolites, and ultimately xenobiotic bioavailability, systemic exposure, efficacy, and toxicity [26,28].

In addition to directly affecting microbial biotransformation, dysbiosis may influence xenobiotic disposition through indirect mechanisms. Altered microbial metabolic activity can modify the abundance of microbial metabolites that compete with xenobiotics for host drug-metabolising enzymes, thereby potentially affecting their activity. Dysbiosis may also alter the intestinal barrier and promote inflammation, thereby influencing xenobiotic absorption and systemic exposure [26,231,232]. In addition, changes in host–microbiome interactions may affect hepatic phase I and phase II detoxification pathways and other processes involved in xenobiotic disposition [26,233]. Therefore, the consequences of dysbiosis may extend beyond the loss or gain of individual microbial metabolic reactions and affect the broader host–microbiome network involved in determining xenobiotic responses.

Dysbiosis is associated with a range of pathological conditions, including inflammatory bowel disease (IBD) [234], diabetes [235], and obesity [236]. Although the specific alterations in microbial composition differ between these conditions, changes in microbial diversity and functional capacity, intestinal barrier integrity, and host–microbiome interactions are common features that may influence the disposition of xenobiotics.

IBD provides an important example of a disease state in which dysbiosis may alter xenobiotic responses. IBD is a chronic inflammatory disorder of the gastrointestinal tract that includes Crohn's disease (particularly affects the terminal ileum) and ulcerative colitis (affects the large intestine, such as the colon) [237,238]. Dysbiosis in IBD has been associated, in cross-sectional studies of patients, with alterations in microbial community structure and metabolic activity, including reductions in *F. prausnitzii* and other members of the Bacillota phylum, and increases in Bacteroidota and Pseudomonadota, including adherent-invasive *E. coli* in Crohn's disease [239,240]. Together with impaired intestinal barrier function and chronic inflammation [241], these alterations may modify microbial xenobiotic transformation, host drug-metabolising enzymes, and transport processes, potentially affecting drug bioavailability, toxicity, and therapeutic outcomes [134].

Similarly, alterations in the gut microbiota have been associated with both type 1 and type 2 diabetes. In type 1 diabetes, impaired intestinal barrier function can increase intestinal permeability and the passage of microbial antigens and microbial products, or even viable microorganisms, across the epithelium [242]. Reported alterations in these patients, all of them compositional rather than functional, include higher levels of *Bifidobacterium* spp., *Clostridium*, Enterobacteriaceae, Clostridiales, Bacteroidales, *R. gnavus*, and

Streptococcus infantarius, and lower levels of Lachnospiraceae, Veillonellaceae, *Streptococcus thermophilus*, and *L. lactis* [242,243]. Additionally, the cathelicidin antimicrobial peptide, expressed in pancreatic β cells, has been associated with protection against type 1 diabetes, and its production is regulated by SCFAs produced by the gut microbiota. This finding supports an interplay between the pancreas and the gut microbiota in protecting against the development of type 1 diabetes [244–246].

Type 2 diabetes is characterised by altered intestinal barrier function, chronic low-grade inflammation, and metabolic disturbances [247,248]. It has been associated with reduced microbial diversity and changes in the abundance of specific microbial taxa, including reductions in the genus *Bifidobacterium*, the phylum Bacillota, and several species of *Clostridium*, together with an increase in the class Betaproteobacteria and several species of *Lactobacillus* [249–251]. These disease-associated changes may influence xenobiotic responses by altering the expression and activity of microbial enzymes involved in xenobiotic biotransformation and by affecting host metabolic processes, including hepatic phase I and phase II detoxification pathways [252].

Obesity is also associated with alterations in gut microbiota composition and microbiome function. The human gut microbiota has been implicated in obesity and has been particularly associated with changes in the relative abundance of the phyla Bacteroidota and Bacillota, with some studies reporting a decrease in Bacteroidota and a proportional increase in Bacillota, first in genetically obese mice [253] and subsequently in obese human subjects [254], although this ratio has not been consistently replicated across studies. Several studies have also reported reduced levels of *Akkermansia*, *Oscillibacter*, *Faecalibacterium*, *Alistipes*, *Lactobacillus*, *Bifidobacterium*, *Methanobrevibacter*, and *Coprococcus*, together with increased levels of specific taxa including *Lactobacillus reuteri*, *Porphyromonas*, *Campylobacter*, *Staphylococcus*, *Bacteroides*, *Dialister*, *Parabacteroides*, and *Ruminococcus* [255,256]. These apparently opposing findings reflect the different taxonomic levels examined, since changes reported for a phylum or genus are not necessarily reproduced at the level of its constituent genera or species. Beyond its metabolic functions, the gut microbiome may also contribute to obesity through host–microbiome interactions affecting brain function and food intake, including the production of microbial metabolites and neuromodulators involved in gastrointestinal and neuroendocrine signalling [257]. Obesity-associated dysbiosis may therefore influence multiple microbiota-mediated functions. In the context of xenobiotic exposure, alterations in the activity of microbial enzymes involved in xenobiotic biotransformation may modify xenobiotic metabolism, while increased intestinal permeability and inflammation may enable greater systemic absorption of microbial metabolites and xenobiotics [258].

Overall, dysbiosis should not be considered solely as a change in the taxonomic composition of the gut microbiota. By altering microbiome functional capacity, dysbiosis can modify multiple processes mediated by the microbiota and affect its interactions with the host [259,260]. Consequently, changes associated with dysbiosis may alter the transformation, absorption, and systemic exposure of xenobiotics and their metabolites, potentially influencing their pharmacological activity, toxicity, and other biological effects. The impact of dysbiosis is therefore likely to depend on the specific microbial functions that are lost, reduced, acquired, or enhanced, as well as on the characteristics of the xenobiotic and the host.

7. Gut Microbiome–Brain Interactions and CNS Effects

In recent years, there has been increasing interest in the gut–brain axis due to its recognised role in the regulation of health and the development of disease [261]. The gut–brain axis is a bidirectional communication network involving multiple biological systems, including the enteric nervous system (ENS), the immune system, and the

enteroendocrine system. Communication occurs through neural, endocrine, immune, and circulatory pathways, enabling signals originating in the gastrointestinal tract to influence the CNS through, among other routes, the vagus nerve, spinal pathways, and the systemic circulation [262]. Conversely, the brain can regulate gastrointestinal motility, homeostasis, and gut microbiota composition through neural and neuroendocrine mechanisms [263].

Through these interconnected pathways, alterations in the gut microbiome, including changes in the overall microbiota composition and functional capacity, may modify neural, endocrine, and immune signalling, affect intestinal and BBB integrity, and alter the production of microbial metabolites [27,213]. These changes may consequently influence neuroinflammatory responses, neurotransmitter-related pathways, and other processes that can modify the susceptibility of the CNS to external insults [17,23,264]. Thus, the gut–brain axis involves several mechanisms through which the gut microbiome may influence susceptibility to xenobiotic-induced neurotoxicity. In addition to indirectly affecting CNS vulnerability through changes in host–microbiome signalling and barrier function, the gut microbiota can directly transform xenobiotics, altering their chemical identity, biological activity, bioavailability, and systemic exposure, as discussed above [14,24,26]. Together, these mechanisms may influence both the amount and the biological activity of xenobiotics and their metabolites reaching the CNS, as well as the susceptibility of neural cells to their toxic effects. An overview of the proposed mechanisms linking gut microbiome alterations, xenobiotic transformation, gut–brain signalling, BBB integrity, and CNS effects is presented in Figure 3.

7.1. Gut–Brain Communication Pathways

7.1.1. Neural Pathway

The autonomic nervous system (ANS), which comprises the sympathetic and parasympathetic nervous systems, forms a neural network that enables bidirectional communication among the different components of the gut–brain axis [265]. The ENS, a component of the peripheral nervous system (PNS) that is regulated by the ANS, is a complex network containing approximately 200 to 600 million neurons distributed throughout the gastrointestinal tract. It controls several functions, playing an essential role in maintaining gut homeostasis and interacting with the gut microbiota [266]. The ENS communicates with the CNS through both parasympathetic pathways, primarily via the vagus nerve, and sympathetic pathways involving prevertebral ganglia.

The vagus nerve is a critical component of the PNS, originating in the medulla oblongata of the brainstem and extending to several major organs, including the heart, lungs, and gastrointestinal tract [210]. It enables bidirectional communication and contributes to the regulation of multiple physiological processes. Approximately 80–90% of vagal nerve fibres are sensory afferent fibres, whereas the remaining fibres are predominantly myelinated efferent fibres [211]. Afferent fibres transmit sensory information from peripheral organs to the brain. Within the gastrointestinal tract, afferent signals can originate from tension receptors in the stomach and oesophagus, as well as mechanoreceptors and chemoreceptors in the gastric mucosa, and are transmitted to the nucleus tractus solitarius in the medulla oblongata via the vagus nerve. Vagal afferent innervation also contributes to metabolic regulation, including the regulation of insulin secretion and glucose concentrations in the pancreas, as well as the sensing of amino acid, fatty acid, and glucose concentrations in the liver and portal vein [210].

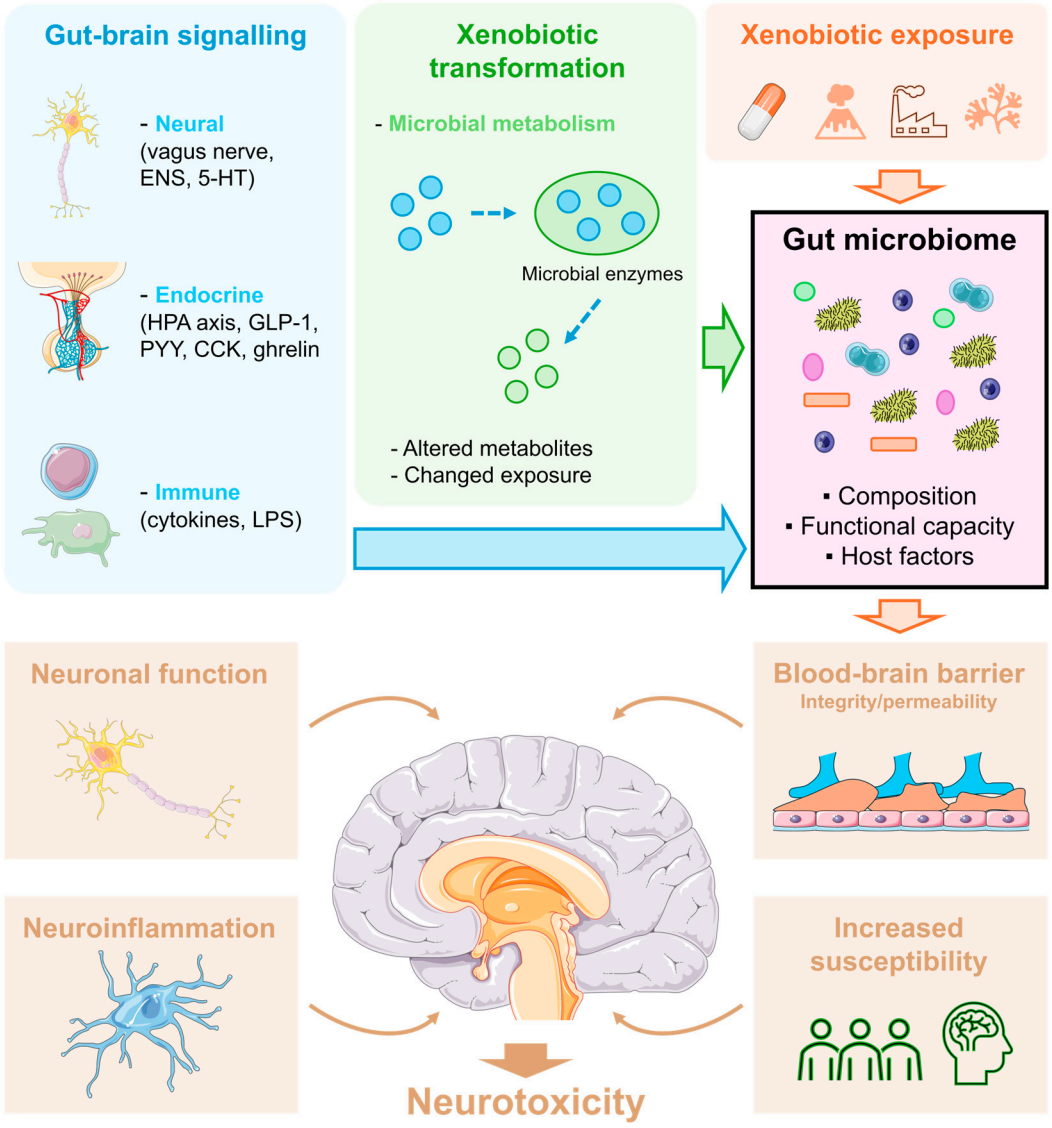

Figure 3. Gut microbiome-mediated pathways linking xenobiotic exposure to neurotoxicity. The gut microbiome can be influenced by gut–brain signalling, xenobiotics, and xenobiotic-derived metabolites, while also contributing to xenobiotic transformation and metabolite production. These interactions may affect blood–brain barrier integrity and permeability. Additional factors, including neuronal function, neuroinflammation, and states of increased individual susceptibility, may contribute to increased vulnerability to neurotoxicity. 5-HT, 5-hydroxytryptamine (serotonin); CCK, cholecystokinin; ENS, enteric nervous system; GLP-1, glucagon-like peptide-1; HPA, hypothalamic–pituitary–adrenal; LPS, lipopolysaccharide; PYY, peptide YY. The illustration includes elements from Servier Medical Art.

In contrast, vagal efferent fibres originate primarily from the nucleus ambiguus and the dorsal motor nucleus of the vagus in the medulla oblongata. The dorsal motor nucleus of the vagus represents the principal parasympathetic efferent signalling pathway of the vagus nerve and plays an important role in regulating intestinal motility, intestinal muscle contraction, and secretion [212]. Together, afferent and efferent vagal pathways form an integrated communication system that contributes to the coordinated regulation of the ANS.

Vagal afferent fibres can be activated by several signals, including serotonin (5-HT) [267]. This neurotransmitter is predominantly synthesised in the gut by enterochromaffin cells and plays an essential role in immune responses, gut motility, and neural regulation

[268]. 5-HT synthesis begins with tryptophan, which is obtained from dietary sources. Enterochromaffin cells take up tryptophan and convert it into 5-hydroxytryptophan (5-HTP) through the action of tryptophan hydroxylase (TPH), primarily the TPH-1 isoform. Subsequently, aromatic *L*-amino acid decarboxylase converts 5-HTP into 5-HT [269]. Several bacterial taxa, including *Lactobacillus*, *Lactococcus*, *E. coli*, *Streptococcus*, and *Klebsiella*, have also been reported to produce 5-HT [270].

The gut microbiota also has an important relationship with ENS development and function. Microbial signals can interact with pattern-recognition receptors, including Toll-like receptors (TLRs) such as TLR2 and TLR4, which recognise microbial components, including LPS, viral RNA, and peptidoglycan [271]. A study in germ-free mice demonstrated impaired ENS structure, reduced numbers of enteric neurons, altered sensory signalling, and changes in gut motility, highlighting the importance of the gut microbiota in ENS development and function [266].

The anatomical position of the ENS gives it a particular toxicological significance that is easily overlooked. It is the first neural structure that an ingested xenobiotic encounters, and it encounters it at luminal concentrations that may exceed those ever reached in the systemic circulation, since no absorptive or hepatic first-pass step intervenes [266]. Enteric neurons and glia express the molecular targets of several classes of neurotoxicants, including acetylcholinesterase [145,146], and are directly susceptible to others, including reactive aldehydes such as acrolein [150,151], which, as discussed in Section 4, may be generated in the lumen by microbial metabolism of glycerol [152]. Injury at this level has a consequence that is specific to the gut: because the ENS governs motility, enteric damage alters transit time and therefore the duration of contact between a xenobiotic and the microbial community [272], which in turn determines the extent of microbial transformation. Microbial activity, enteric function and xenobiotic exposure are thus coupled in both directions rather than arranged in a simple sequence. Enteric involvement may also precede central involvement in disease. In Parkinson's disease, gastrointestinal symptoms and enteric α-synuclein aggregation are frequently observed years before motor signs appear [273,274], as discussed in Section 7.3. For the purposes of this review, the ENS should therefore be regarded both as a target of xenobiotic-induced neurotoxicity in its own right and as a determinant of the luminal conditions under which microbial transformation takes place.

In addition, the gut microbiota can produce or influence the availability of other neuroactive compounds, including γ-aminobutyric acid (GABA) and dopamine [267], as well as SCFAs [275], thereby further influencing ENS activity. SCFAs, particularly butyrate, acetate, and propionate, are produced through bacterial fermentation and contribute to gut health through several local effects, including the stimulation of mucus production, maintenance of intestinal barrier integrity, and protection against inflammation [214,276]. These metabolites can also exert anti-inflammatory effects by influencing the recruitment and migration of immune cells, the expression of inflammatory cytokines and chemokines, and the differentiation of B and T cells [277].

SCFAs have been reported to act through two main cellular mechanisms. They can bind to and activate G protein-coupled receptors (GPCRs) expressed in different cell types, including enteroendocrine cells, thereby stimulating the secretion of glucagon-like peptide-1 (GLP-1) and peptide YY. Alternatively, they can inhibit histone deacetylase activity, promoting the acetylation of lysine residues in nucleosomal histones [272,278,279]. SCFAs can also interact with neural pathways by affecting the levels of neurotrophic factors, reinforcing BBB integrity, promoting 5-HT biosynthesis, and directly activating vagal afferent pathways [280]. In particular, propionate and butyrate can influence potassium levels [281], whereas acetate can alter the levels of neurotransmitters, including glutamine, glutamate, and GABA, in the hypothalamus [282]. In addition, SCFAs can regulate the expression of several enzymes involved in neurotransmitter biosynthesis, including TPH-

1 and tyrosine hydroxylase, which is involved in the synthesis of dopamine, noradrenaline, and adrenaline [283–286].

Collectively, these findings indicate that the neural pathway provides a rapid, bidirectional route through which microbial activity can influence CNS function and, potentially, the neurotoxic response to xenobiotics.

7.1.2. Endocrine Pathway

In parallel with neural signalling, the gut microbiota communicates with the CNS through endocrine mechanisms, acting on enteroendocrine cells and modulating the hypothalamic–pituitary–adrenal (HPA) axis. The hormones and peptides released through these pathways reach the brain via the systemic circulation and participate in the physiological response to stress. The HPA axis is an essential neuroendocrine component of the gut–brain axis. During stress, the hypothalamus synthesises and releases corticotropin-releasing hormone, which acts on the anterior pituitary gland and stimulates the release of adrenocorticotropic hormone (ACTH) into the systemic circulation. ACTH subsequently acts on the adrenal glands, stimulating the synthesis and release of glucocorticoids, which serve as downstream effectors of the HPA axis [287,288]. Studies have shown that modulation of the gut microbiota through the administration of probiotics and prebiotics can attenuate stress-induced increases in corticosterone levels [289,290].

Enteroendocrine cells, which are distributed throughout the gastrointestinal tract, are also fundamental for bidirectional communication between the gut and the brain. These cells can be classified according to the hormones they secrete, including K cells, which produce glucose-dependent insulinotropic polypeptide; I cells, which secrete cholecystokinin (CCK); and L cells, which primarily secrete GLP-1 [215]. Enteroendocrine cells exhibit high expression of chemosensory components, including GPCRs and nutrient transporters, and their specialised configuration enables them to detect changes in the gut lumen. Several gut hormones, including GLP-1, peptide YY, CCK, and ghrelin, are involved in multiple aspects of gut–brain communication [291]. For example, GLP-1 has been reported to improve hippocampal synaptic plasticity, attenuate neuroglial cell activation, and improve motor function, memory, and learning [292]. Similarly, ghrelin is involved in glucose and lipid metabolism but has also been associated with improved learning and memory consolidation [293]. In addition to their endocrine functions, L cells can enable rapid gut-to-brain signalling through direct synaptic connections with the ENS via neuropods, further highlighting the role of enteroendocrine cells in gut–brain communication [294].

Although much of this evidence derives from experimental models, it indicates that microbiota-driven modulation of enteroendocrine and HPA-axis signalling represents an additional route by which the gut microbiome may influence CNS vulnerability to xenobiotics.

7.1.3. Immune Pathway

A third route of gut–brain communication is immunological. The immune system plays a crucial role in the gut–brain axis, acting as an important mediator of signals originating from the gut microbiota and contributing to neuroimmune responses that can influence brain function and behaviour. It is well known that the immune system plays an important role in shaping the composition of the gut microbiota, while the microbiota, in turn, contributes to normal immune development and regulates immune responses [295]. Studies using germ-free mice have demonstrated that the acquisition of a gut microbiota is closely associated with immune system development. Animals lacking a microbiota exhibit reduced numbers of lymphocytes, decreased immunoglobulin A (IgA) production, and immature gut-associated lymphoid tissues [296–298].

The gut microbiota can modulate the development and function of both innate and adaptive immune responses, thereby influencing neuroinflammatory processes. In the CNS, microglia and astrocytes represent important components of innate immune responses, whereas B and T cells are key components of adaptive immunity [216]. Microglia are the resident macrophages of the brain and are involved in the regulation of synaptic transmission, maintenance of homeostasis, and neuronal circuit formation [299]. Studies using germ-free mice have demonstrated increased numbers of immature microglia in different brain regions. However, administration of SCFAs was sufficient to promote microglial maturation, supporting an important role for microbiota-derived metabolites in microglial development [300,301].

The gut microbiota can also interact with astrocytes through the activation of the aryl hydrocarbon receptor (AhR) by microbial metabolites. This interaction can promote an anti-inflammatory state by inhibiting the recruitment of neurotoxic immune cells through type I interferon signalling [16]. Regarding adaptive immunity, B cells play an important role in mucosal immunity, and alterations in B-cell function can consequently affect the intestinal microbiota. T cells also contribute to the maintenance of host–microbiota interactions, particularly regulatory T cells, which can contribute to the diversification of the gut microbiota by promoting the growth of species belonging to the Bacillota phylum, regulating IgA selection in Peyer's patches, and suppressing inflammation [295].

The activation of immune cells can trigger a cascade of events involving the release of inflammatory mediators, cytokines, and chemokines, which act as chemical messengers facilitating communication between the immune system, the gut microbiota, and the CNS [262]. Excessive or persistent cytokine production can increase systemic inflammatory signalling and affect brain function. When the microbiota is dysregulated, alterations in intestinal barrier integrity may facilitate the translocation of microbial components and metabolites into the systemic circulation. In addition, dysbiosis and systemic inflammation may compromise BBB integrity, potentially facilitating the interaction of circulating inflammatory mediators and microbial-derived molecules with the CNS. These processes can activate resident immune cells, including microglia, thereby promoting a pro-inflammatory state in the brain [267,302].

7.1.4. Gut-Derived Neurotransmitters as a Distinct Class of Mediators

SCFAs and bile acids are the microbial mediators most often discussed in relation to the gut–brain axis, but they are not the only ones. The gut microbiota also produces, or regulates host production of, molecules that are themselves neurotransmitters, and these warrant treatment as a class rather than as an appendix to the SCFA literature. Their defining feature, from a toxicological standpoint, is a dissociation between where they are produced and where they act. Most are synthesised in the lumen or the mucosa and do not themselves cross the blood–brain barrier to any appreciable extent, yet they influence central function through vagal, endocrine and immune routes [303,304]. Attributing their effects to direct central action is a common error in this literature, and the distinction is made explicit for each mediator in Table 1.

5-HT is the clearest example. Approximately 90 per cent of total body 5-HT is synthesised peripherally, chiefly by intestinal enterochromaffin cells [268,269], and its production is stimulated by microbial products, notably butyrate and secondary bile acids [285], as well as by spore-forming members of the microbiota [286]. This peripheral pool is functionally distinct from the central one and does not supply it, since 5-HT does not cross the BBB [270]. Its central consequences are mediated by 5-HT3 receptors on vagal afferents [210,268], as described in Section 7.1.1.

Table 1. Microbially produced or microbially regulated mediators of gut–brain communication, with the taxa principally responsible, the host target, whether the mediator itself reaches the CNS in appreciable amounts, the reported central or behavioural effect and the level of evidence.

Mediator (Class)	Principal Producing or Modulating Taxa	Host Target or Receptor	Appreciable BBB Penetration?	Reported CNS or Behavioural Effect	Level of Evidence	References
Butyrate (short-chain fatty acid)	*F. prausnitzii*, *Roseburia* spp., *Eubacterium* spp.	FFAR2/FFAR3 (GPR43/GPR41); inhibition of histone deacetylases	Limited; monocarboxylate transporter-mediated	Increased tight-junction protein expression and reduced BBB permeability; microglial maturation	Established (germ-free and gnotobiotic mice)	[275,276,278–280,305]
Acetate and propionate (short-chain fatty acids)	*Bacteroides* spp., *Prevotella* spp., *Akkermansia muciniphila*	FFAR2/FFAR3 on enteroendocrine and immune cells	Low	Appetite regulation, HPA-axis modulation, microglial homeostasis	Mechanistic (animal)	[275,278–280,282]
Secondary bile acids	*Clostridium* spp. and *Bacteroides* spp. (7-α-dehydroxylation)	Farnesoid X receptor; TGR5	Limited	Enteroendocrine signalling; modulation of neuroinflammation	Mechanistic (animal, in vitro)	[69,70,72,166]
γ-aminobutyric acid (neurotransmitter)	*L. brevis*, *Bifidobacterium dentium* (a minority of intestinal strains)	GABA-A and GABA-B receptors on enteric neurons and vagal afferents	Negligible at physiological concentrations	Behavioural effects abolished by vagotomy, indicating an indirect route	Mechanistic (animal)	[306–308]
Serotonin (neurotransmitter)	Host enterochromaffin cells under microbial and SCFA control; spore-forming bacteria	5-HT3 receptors on vagal afferents	Does not cross; the peripheral pool is distinct from the central pool	Motility and vagal afferent activation	Established (animal); human evidence associative	[210,269,270,285,286]
Dopamine and noradrenaline (neurotransmitters)	*Clostridium* spp. and other β-glucuronidase-producing taxa; deconjugation of host conjugates (not biosynthesis)	Enteric adrenergic and dopaminergic receptors	Negligible	Enteric signalling; luminal consumption of levodopa (see Table 2)	Mechanistic (in vitro, animal)	[117,120,309,310]
Histamine (biogenic amine)	*L. reuteri* (bacterial histidine decarboxylase)	H2 receptor on mucosal immune cells	Negligible	Mucosal immune modulation	Mechanistic (in vitro, animal)	[311]
Indole and tryptophan derivatives	*Lactobacillus* spp., *Clostridium* spp., *Peptostreptococcus* spp.	Aryl hydrocarbon receptor on astrocytes and microglia	Yes	Anti-inflammatory astrocyte phenotype via type I interferon signalling	Established (animal)	[16,267,312]
Lipopolysaccharide and other microbial components	Gram-negative taxa	TLR2 and TLR4	Not as the intact molecule; acts through systemic inflammation	Increased BBB permeability (approximately 60 per cent in mice)	Established (animal)	[219,271,302]

BBB, blood–brain barrier; FFAR, free fatty acid receptor; GABA, γ-aminobutyric acid; HPA, hypothalamic–pituitary–adrenal; 5-HT, 5-hydroxytryptamine (serotonin); SCFA, short-chain fatty acid; TGR5, Takeda G protein-coupled receptor 5; TLR, Toll-like receptor.

GABA is produced by a restricted set of intestinal Lactobacillus and Bifidobacterium strains (of 91 human intestinal isolates screened, only one Lactobacillus and four Bifidobacterium strains were producers, with *L. brevis* and *B. dentium* the most efficient [306]) and acts on GABA-A and GABA-B receptors expressed by enteric neurons, vagal afferents and immune cells [307,308]. In rodents, administration of *Lactobacillus rhamnosus* JB-1 modifies central GABA receptor expression and anxiety- and depression-related behaviours through mechanisms dependent on the vagus nerve. However, the specific contribution of bacterially produced GABA to these effects has not been established [313].

Dopamine and noradrenaline illustrate the point most clearly of all. In germ-free mice, luminal catecholamines are present almost entirely in a biologically inactive, conjugated form, whereas in conventionally colonised animals, they are free and active [309]. Association of germ-free mice with *Clostridium* species or with a conventional faecal flora, both rich in β-glucuronidase activity, raises free catecholamine levels sharply, and an *Escherichia coli* strain lacking the β-glucuronidase gene fails to do so. The microbial contribution here is therefore not biosynthesis but deconjugation (the same β-glucuronidase chemistry that regenerates SN-38 from its glucuronide [83], described in Section 3.1) applied to an endogenous signalling molecule rather than to a drug [309].

Histamine is generated from dietary histidine by bacterial histidine decarboxylase, characterised in *L.s reuteri*, and acts on the H2 receptor of mucosal immune cells, where it suppresses tumour necrosis factor production through cyclic adenosine-5′-monophosphate and protein kinase A signalling [311].

These mediators are relevant to the present review in two ways. First, they modulate the immune and barrier states that determine CNS vulnerability. 5-HT suppresses Th1 and Th17 responses [312], GABA modulates interleukin-17 and interferon-γ production by T cells [314,315], and dopamine acts on both T cells and microglia, with opposing effects at D1-like and D2-like receptors [210,310]. Microbial perturbation of these signals therefore alters the inflammatory context into which a neurotoxicant is delivered, rather than altering the neurotoxicant itself. Second, and more directly, the same microbial enzymes that generate or liberate these neurotransmitters act on xenobiotics. The tyrosine decarboxylase that converts levodopa to dopamine is the same class of enzyme responsible for microbial production of biogenic amines [120]. The distinction between a microbial transformation of an endogenous signalling molecule and a microbial transformation of a drug is, at the level of enzyme chemistry, often no distinction at all.

Collectively, the neural, endocrine, and immune pathways described above, together with the gut-derived neurotransmitters that traverse them, provide interconnected mechanisms through which the gut microbiome can influence CNS function. Microbial metabolites and components, together with microbiota-induced changes in host signalling, can modulate neuronal activity, neuroendocrine responses, immune homeostasis, and neuroinflammatory processes. Through these interactions, alterations in the gut microbiome may contribute to changes in CNS physiology and modify the susceptibility of neural cells to external insults, including xenobiotic-induced neurotoxicity.

7.2. Influence of the Gut Microbiome on Blood–Brain Barrier Integrity

The BBB is a specialised interface that separates the CNS from the systemic circulation and tightly regulates the exchange of molecules between these compartments. Its integrity is maintained by tight junction complexes between endothelial cells, which include transmembrane proteins such as occludin, junctional adhesion molecules, and claudins, together with the actin cytoskeleton [218]. Disruption of BBB integrity has been associated with several neurological and neurodegenerative disorders, as well as neurotoxic outcomes [217]. Microbial metabolites and products, including LPS, vitamins, SCFAs, and

TMA and its N-oxide metabolite TMAO, have been shown to modulate BBB permeability [219].

SCFAs can reach the brain and influence BBB integrity and permeability, particularly through the modulation of tight junction proteins. A study in germ-free mice demonstrated that the administration of sodium butyrate improved BBB integrity by increasing the expression of tight junction proteins and reducing BBB permeability [213]. One proposed mechanism underlying this protective effect involves the interaction of SCFAs with GPCRs expressed on endothelial cells [305].

The systemic immune response also plays an important role in the maintenance of BBB integrity, and excessive immune activation can contribute to its disruption. For example, systemic administration of LPS to mice increased BBB permeability by approximately 60%, potentially facilitating the entry of circulating compounds, including xenobiotics and their metabolites [219].

Thus, alterations in the gut microbiota and its metabolic activity may influence BBB integrity through the production of microbial metabolites and immune-mediated mechanisms. Changes in BBB permeability may, in turn, modify CNS exposure to xenobiotics, their metabolites, and other circulating compounds, representing a potentially important mechanism linking gut microbiome-mediated xenobiotic metabolism to neurotoxicity.

Whether a microbial transformation is toxicologically relevant for the CNS depends on quantitative considerations that are frequently omitted from mechanistic descriptions. A microbial reaction that is readily demonstrable in vitro will only alter central exposure if the metabolite is formed in the lumen at concentrations that are meaningful relative to the administered dose, if an appreciable fraction escapes luminal degradation and is absorbed across the intestinal epithelium, if it survives first-pass hepatic metabolism, and if the unbound plasma fraction is sufficient to permit transfer across the BBB, either by passive diffusion, which favours small, lipophilic and poorly ionised species, or by carrier-mediated transport. Efflux at the barrier, principally by P-gp and breast cancer resistance protein, may offset influx entirely. Few of the examples discussed above satisfy all of these conditions with measured data. Luminal and portal concentrations are rarely reported, and CNS concentrations of microbially derived metabolites are almost never measured. For this reason, a distinction should be drawn between effects that are exerted within the gut lumen or at the epithelium, such as the SN-38-mediated intestinal injury produced by bacterial β-glucuronidases, and effects that require systemic distribution and central penetration, such as those proposed for microbially generated acrolein or for arsenic species. In the present state of the evidence, the former are demonstrated, whereas the latter remain, in most cases, quantitatively unproven and should be read as plausible mechanisms rather than as established routes of microbiome-mediated neurotoxicity.

7.3. Gut Microbiome-Mediated Modulation of Neurotoxicity and Neurological Disorders

Three distinct types of evidence are brought together in this section, and it is important to keep them apart. The first is direct demonstration that the gut microbiota chemically transforms a xenobiotic, with an identified enzyme, taxon or metabolite. The second is the demonstration that such a transformation alters therapeutic efficacy or systemic exposure, which is a pharmacological rather than a toxicological outcome. The third is an observed association between microbiota composition and a neurological disease/neurotoxicity, which establishes neither transformation nor causation. Only the first two rest on experimental manipulation. The third is, in nearly all cases, cross-sectional. Table 2 applies this distinction compound by compound, and the text below follows the same order, moving from transformations that have been demonstrated to associations that have not.

Table 2. Xenobiotics for which a gut microbial transformation has been characterised, with the corresponding microbial pathway, metabolite, experimental model, reported endpoint, level of evidence and principal limitations. "Established" denotes reproducible demonstration in the stated system, including human data where indicated; "mechanistic" denotes demonstration in vitro or in animals without human confirmation; "plausible mechanism" denotes a reaction that is chemically expected but for which the toxicological consequence has not been demonstrated.

Xenobiotic (Class)	Microbial Reaction/ Enzyme/ Taxa	Main Metabolite(s)	Experimental Model	Neurological or Toxicological Endpoint	Level of Evidence	Principal Limitations	References
Sulphasalazine (drug)	Azo-bond reduction; bacterial azoreductases; colonic anaerobes	5-ASA + sulphapyridine	Human (therapeutic use); in vitro	Local colonic anti-inflammatory activity; no CNS endpoint	Established (human)	Prototype of bioactivation; no neurological outcome assessed	[132,133]
Prontosil (drug)	Azoreduction; azoreductases	Sulphanilamide	Rat; in vitro	Antibacterial activation; no CNS endpoint	Established (animal, in vitro)	Historical data; no modern quantification	[138,139]
Irinotecan/ SN-38G (drug)	Deconjugation; bacterial β-glucuronidases (e.g., *E. coli*)	SN-38 (regenerated active metabolite)	Mouse; human clinical correlation	Severe intestinal toxicity and epithelial barrier disruption; CNS effect indirect	Established (human toxicity; animal mechanism)	Effect is luminal; increased CNS exposure to other agents is inferred, not measured	[58,78,142]
Digoxin (drug)	Reduction in the α, β-unsaturated lactone; cardenolide reductase (cgr operon); *E. lenta*	Dihydrodigoxin (inactive)	Human faecal samples; gnotobiotic mice	Loss of therapeutic efficacy; no CNS endpoint	Established (human and animal)	Strain-dependent; relates to efficacy, not to neurotoxicity	[99]
Levodopa (drug)	Decarboxylation by tyrosine decarboxylase (*E. faecalis*), then dehydroxylation by dopamine dehydroxylase (*E. lenta*)	Dopamine, then m-tyramine	In vitro; human faecal communities; mice	Reduced levodopa bioavailability and reduced CNS delivery; altered therapeutic response	Strong mechanistic (in vitro, animal); human evidence associative	Affects efficacy rather than neurotoxicity; individual contribution not quantified in vivo	[120]
Sorivudine (with 5-FU prodrugs)	Hydrolysis of the glycosidic bond; intestinal anaerobes (*Bacteroides* spp.)	(E)-5-(2-bromovinyl)uracil; irreversible DPD inhibition	Human (fatal clinical interactions); rat	Lethal 5-FU toxicity, including neurotoxicity	Established (human)	CNS effects are secondary to systemic 5-FU accumulation, not to the microbial metabolite itself	[62,63]
Chlorpyrifos (pesticide)	Oxidative desulfuration; host CYP450 and gut bacteria (*L.s lactis* converted 61% and *E. coli*	Chlorpyrifos-oxon	Bacterial cultures in vitro; rodent	Acetylcholinesterase inhibition—a direct neurotoxic mechanism	Mechanistic (in vitro, animal)	Relative contribution of microbial versus hepatic activation not	[145,146]

	16% of chlorpyrifos to the oxon in vitro)					quantified in vivo; no human data	
Inorganic arsenic (metalloid)	Reduction, methylation and thiolation; sulfate-reducing bacteria	MMA(III), DMA(III), thioarsenicals	Colon simulators in vitro; rodent	Arsenic is an established neurotoxicant; speciation determines potency	Mechanistic (in vitro, animal)	CNS concentrations of microbially generated species not measured	[144]
Glycerol (dietary substrate)	Vitamin B12-dependent glycerol/diol dehydratase; *Eubacterium hallii*, *Blautia obeum*, *R. gnavus*, *Flavonifractor plautii*, *Intestinimonas butyriciproducens*, *Veillonella* spp. *E. coli*, *Klebsiella* spp., *Citrobacter* spp. (~11% of gut microbiota predicted to carry the enzyme)	3-Hydroxypropionaldehyde (reuterin), which dehydrates to acrolein	In vitro; mouse	Acrolein causes oxidative stress and axonal injury	Mechanistic (in vitro, animal)	Transfer of luminal acrolein to the CNS not demonstrated	[152]
Heterocyclic amines (IQ, PhIP, MeIQx)	Deconjugation by β-glucuronidase; glycerol/diol dehydratase	Reactivated N-hydroxy derivatives	Gnotobiotic rat	Colonic genotoxicity; no CNS endpoint	Established (animal)	Genotoxic rather than neurotoxic endpoint	[91,155]
Azo and nitro compounds (e.g., nitrazepam, 2,4,6-trinitrotoluene)	Azo- and nitroreduction	Reactive arylamine intermediates	In vitro; rodent	Protein and DNA adduct formation; neurotoxicity proposed	Plausible mechanism only	No direct CNS evidence	[24,147,153,154]

5-ASA, 5-aminosalicylic acid; 5-FU, 5-fluorouracil; CNS, central nervous system; CYP450, cytochrome P450; DMA, dimethylarsinous acid; DPD, dihydropyrimidine dehydrogenase; IQ, 2-Amino-3-methylimidazo[4,5-f]quinoline; MeIQx, 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline; MMA, monomethylarsonous acid; PhIP, 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38 (7-ethyl-10-hydroxycamptothecin); SN-38G, SN-38 glucuronide.

Although the gut–brain communication pathways described above can influence CNS homeostasis and susceptibility to external insults, gut microbiome-mediated xenobiotic transformation may provide an additional mechanism through which neurotoxic outcomes are modified. As discussed in Section 4, microbial metabolism can alter the chemical identity and biological activity of xenobiotics [26,30]. When these transformations involve neuroactive or neurotoxic compounds, they may consequently modify exposure and the potential for adverse effects on the nervous system.

One of the clearest examples of this mechanism is chlorpyrifos. This organophosphate pesticide is converted by host cytochrome P450 enzymes, and also by certain gut bacteria, to chlorpyrifos-oxon, a substantially more potent inhibitor of acetylcholinesterase than the parent compound [145,146]. Experimental studies indicate that the microbiota can therefore act directly on this bioactivation step. In *Drosophila melanogaster*, *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) isolated from the gut was shown to metabolise chlorpyrifos preferentially to chlorpyrifos-oxon, and conventionally colonised animals exhibited greater chlorpyrifos toxicity than germ-free or antibiotic-treated animals [316]. In addition, chlorpyrifos exposure has been reported to reduce *Lactobacillus* spp. and to alter the expression of claudin-5, zonula occludens-1, and transient receptor potential canonical 4, compromising BBB integrity.

Microbial transformations may also influence neurotoxicity by modifying xenobiotic speciation. Arsenic represents a relevant example, as gut microorganisms can transform inorganic arsenic to distinct chemical forms with different toxic potential [143,144]. Importantly, arsenic is a well-established neurotoxicant, and exposure to toxic arsenic species can induce neurological effects through mechanisms including oxidative stress, mitochondrial dysfunction, and disruption of neuronal signalling [317,318]. Therefore, microbiome-mediated changes in arsenic metabolism and speciation may modify the amount and type of arsenic species available for systemic absorption and distribution to the nervous system, potentially influencing susceptibility to arsenic-induced neurotoxicity.

The gut microbiome may also modify host exposure to compounds acting directly on the nervous system. For example, as mentioned, gut bacteria can catalyse the conversion of levodopa to dopamine in the intestinal lumen [117,120]. This premature intestinal conversion can reduce the amount of levodopa available for systemic absorption and consequently alter its availability to the CNS. Although this mechanism primarily affects therapeutic efficacy rather than representing a direct neurotoxic effect, it illustrates how microbial metabolism can influence exposure to xenobiotics with neurological targets.

In addition, microbial metabolic activity may contribute to the formation of reactive compounds with recognised neurotoxic potential. As discussed in Section 4, the gut microbiome can contribute to acrolein formation through the metabolism of glycerol [152]. This highly reactive α,β-unsaturated aldehyde is also generated during the metabolic activation of cyclophosphamide by host metabolic enzymes and is widely recognised as an important mediator of cyclophosphamide-induced toxicity [148,149,319]. Its high electrophilicity enables irreversible interactions with cellular proteins and other macromolecules, disrupting cellular redox homeostasis and signalling pathways and promoting oxidative stress, mitochondrial dysfunction, and cellular injury [150,151]. These mechanisms are particularly relevant to the nervous system, where acrolein has been implicated in neuronal and glial injury and in the pathophysiology of several neurological conditions [150,151]. Consequently, differences in microbial metabolic capacity may influence local and potentially systemic exposure to acrolein, thereby modulating susceptibility to its toxic effects, including those affecting the nervous system.

Collectively, these examples demonstrate that microbiome-mediated xenobiotic metabolism can influence neurotoxicity by modifying the formation of neurotoxic metabolites, xenobiotic speciation, exposure to neuroactive compounds, or the production of

reactive molecules capable of damaging neural cells. Thus, the toxicological properties of the parent compound alone may not necessarily reflect the biologically relevant exposure to the nervous system, as microbial metabolism can generate chemically and biologically distinct products [26,30]. Interindividual differences in the gut microbiota composition and microbiome functional capacity may therefore contribute to variability in susceptibility to xenobiotic-induced neurotoxicity by influencing which microbial transformations occur following exposure [30,209]. The strength of this evidence should not be overstated, and its distribution is uneven in a way that matters. Where human data exist, they concern pharmaceuticals. The inactivation of digoxin by *E. lenta* [107,108] and the variable bioavailability of levodopa [49,50] are all documented in people. For environmental neurotoxicants, the position is different. The microbial activation of chlorpyrifos [145], the speciation of inorganic arsenic [144]and the luminal generation of acrolein [152] have been demonstrated in bacterial culture, in invertebrate models or in rodents, but no study has yet shown in humans that microbial transformation of an environmental neurotoxicant measurably alters central nervous system exposure and thereby causes neurotoxicity. The intervening quantitative steps set out in Section 7.2 have generally not been measured. These mechanisms should therefore be read as biologically plausible and experimentally supported, but not as established routes of human neurotoxicity. Establishing them is among the research priorities identified in Section 11.

The relevance of gut microbiome–CNS interactions is also supported by evidence from neurological and neurodegenerative disorders. Although these conditions are not equivalent to xenobiotic-induced neurotoxicity, alterations in gut microbiota composition and microbial activity have been associated with disease processes involving several aspects of CNS dysfunction and pathology [320]. Thus, these disorders provide further evidence for the potential consequences of disturbances in the gut–brain axis for neuronal homeostasis. These disorders are considered here to illustrate the mechanisms by which the CNS becomes vulnerable (barrier dysfunction, neuroinflammation and altered neurotransmitter metabolism) since it is upon such states that a xenobiotic insult is superimposed. They are not presented as evidence that xenobiotics cause these diseases, and the compositional associations described below should be read with the caveats set out at the opening of this section.

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease, with age representing its main risk factor. It is characterised by multiple pathological mechanisms, including amyloid-β (Aβ) deposition, the formation of neurofibrillary tangles composed of hyperphosphorylated tau, mitochondrial dysfunction, oxidative stress, and neuroinflammation [321]. Both experimental models and clinical studies have reported alterations in gut microbiota composition in AD, including decreased abundances of Bacillota, *Bifidobacterium*, *Dialister*, and *A. rectalis,* together with increased abundances of Bacteroidota, *Escherichia*, and *Shigella* [264,322,323] (Figure 4). These alterations have also been associated with changes in microbial metabolic activity, including reduced SCFA availability. In amyloid precursor protein (APP)/presenilin 1 (PS1) mice, reduced SCFA levels in both faeces and brain were correlated with cognitive decline, increased glial activation, and synaptic dysfunction [324]. Butyrate has also demonstrated protective effects against microglia-mediated inflammation in transgenic AD mouse models, with supplementation with *Clostridium butyricum* enhancing these effects [324].

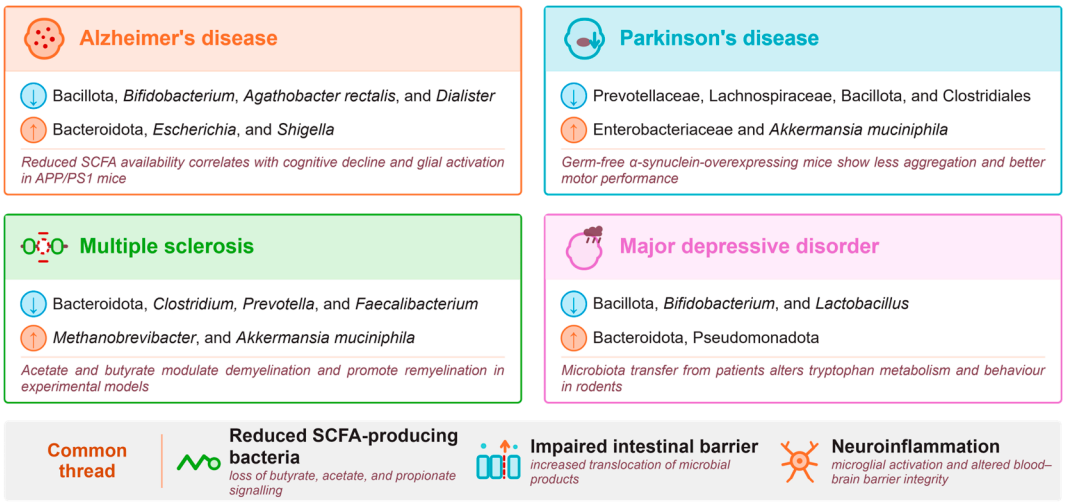

Figure 4. Gut microbiota alterations associated with neurological and neurodegenerative disorders. Overview of reported changes in gut microbial taxa and shared microbiota-related mechanisms across Alzheimer's disease, Parkinson's disease, multiple sclerosis, and major depressive disorder. Common features include reduced short-chain fatty acid production, impaired intestinal barrier integrity, and neuroinflammation. SCFA, short-chain fatty acid; APP, amyloid precursor protein; PS1, presenilin 1. The illustration includes elements from Servier Medical Art.

PD is a neurodegenerative disorder characterised by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. The pathological mechanisms associated with PD include Lewy body formation, neuroinflammation, excitotoxicity, and mitochondrial dysfunction, among others [325,326]. Gastrointestinal alterations are frequently observed in patients with PD and may precede the development of motor symptoms by several years. These alterations may be associated with ENS dysfunction resulting from early α-synuclein aggregation, chronic local inflammation, and increased intestinal permeability [273,274]. Alterations in gut microbiota composition have also been associated with PD progression. PD patients have been reported to exhibit reduced abundances of Prevotellaceae, Lachnospiraceae, Bacillota, and Clostridiales, together with increased levels of Enterobacteriaceae and *A. muciniphila* [327–329] (Figure 4). The reduction in SCFA-producing bacteria in PD patients may contribute to lower SCFA availability, potentially contributing to impaired intestinal barrier integrity and consequent intestinal dysfunction and altered inflammatory responses. In vivo, germ-free mice overexpressing human α-synuclein showed lower microglial activation, decreased α-synuclein aggregation, and improved motor performance compared with colonised mice, further supporting a role of the gut microbiota in PD-related processes [17]. Importantly, gut microbiome-mediated metabolism can also influence the pharmacokinetics and clinical efficacy of levodopa, as previously discussed [117,120].

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease in which impaired oligodendrocyte regeneration and myelin damage contribute to axonal degeneration. Its pathophysiology involves oxidative stress, neuroinflammation, and mitochondrial dysfunction, among other mechanisms [267,330,331]. Alterations in gut microbiota composition have been reported in patients with MS, including reduced levels of Bacteroidota, *Clostridium*, *Prevotella*, and *Faecalibacterium*, whereas increased abundances of *Methanobrevibacter* and *A. muciniphila* have been frequently observed [332–335] (Figure 4). SCFAs have also been implicated in disease progression through their effects on immune regulation. Experimental studies further support a role for the gut microbiota in disease severity. Germ-free mice exhibited attenuated MS-like symptoms, whereas faecal microbiota transplantation from patients with MS resulted in more severe experimental

autoimmune encephalomyelitis and reduced numbers of anti-inflammatory Treg cells compared with transplantation from healthy donors [336,337]. Gut-derived neurotransmitters have been implicated specifically in this setting. 5-HT suppresses the Th17 responses central to demyelination, and dopaminergic signalling modulates interleukin-17 production and microglial activation [267]. MS therefore illustrates, more directly than the other disorders considered here, how the mediators described in Section 7.1.4 may shape the inflammatory state of the CNS.

Alterations in the gut–brain axis have also been associated with major depressive disorder (MDD), a complex and debilitating disorder characterised by alterations in neurotransmitter systems, reduced neuroplasticity, impaired neurogenesis, and abnormal neuronal circuitry [338,339]. Inflammation, HPA-axis dysregulation, alterations in gut microbiota composition, and changes in immune activation have all been reported in patients with MDD [340]. Reported microbial alterations include increased levels of Bacteroidota and Pseudomonadota and decreased abundances of Bacillota, *Bifidobacterium*, and *Lactobacillus* [341,342] (Figure 4). In animal studies, transplantation of gut microbiota from patients with depression induced depression-like behaviours and disrupted tryptophan metabolism, with potential consequences for 5-HT synthesis [343].

Collectively, the evidence from neurological and neurodegenerative disorders further illustrates the importance of gut microbiome–CNS interactions in processes relevant to neuronal homeostasis and disease. Although these disorders are distinct from xenobiotic-induced neurotoxicity and should not be considered direct models of toxicant-induced neurological injury, they demonstrate how disturbances in gut microbiome–brain interactions can influence biological mechanisms involved in CNS dysfunction. Together with the evidence discussed above on microbiome-mediated xenobiotic metabolism, these findings support the concept that the gut microbiome may influence neurological outcomes through both direct modification of xenobiotic exposure and broader effects on biological processes relevant to CNS homeostasis and vulnerability.

8. Emerging Technologies for Studying Gut Microbiome–Xenobiotic Interactions

Understanding how the gut microbiome modulates xenobiotic disposition and toxicity requires tools capable of capturing (i) microbiota composition, (ii) functional activity, and (iii) host response in an integrated and time-resolved manner. As such, the study of microbiome–xenobiotic interactions has rapidly progressed from descriptive microbiome profiling to mechanistic studies at the system level.

This transition has been driven by advances in functional genomics, multi-omics technologies, high-throughput sequencing, advanced experimental models, and computational biology. Together, these approaches enable us to move from identifying exposure-associated changes in microbial communities towards determining which microbial functions transform xenobiotics, which metabolites are generated, how these transformations alter host exposure, and whether microbiome-associated changes contribute causally to toxicity. Importantly, no single technology can resolve all these dimensions. The major advance therefore lies in their integration across complementary experimental scales.

8.1. Microbiome Profiling and Metagenomics

The first step in investigating microbiome–xenobiotic interactions is to characterise the microbial community exposed to the xenobiotic. Amplicon sequencing, particularly 16S ribosomal RNA sequencing, has been extensively used to determine changes in bacterial composition and relative abundance [344,345]. Although relatively inexpensive and technically accessible, this approach provides limited information about microbial

functional capacity [222] and does not directly identify the enzymes responsible for xenobiotic transformation [346].

Shotgun metagenomic sequencing provides substantially greater functional resolution by simultaneously characterising microbial genomes and their encoded genes [347]. This is particularly relevant to xenobiotic toxicology, as microbial transformation depends on specific enzymes and metabolic pathways rather than on taxonomy alone. Metagenomic data can therefore be explored for genes potentially involved in reactions responsible for the chemical modification of xenobiotics [24].

Metagenomics has also provided evidence that exposure to environmental chemicals can exert selective pressure on microbial communities. For example, a large human study published in 2024 showed that exposure to environmental pollutants was associated with enrichment of microbial functions related to xenobiotic degradation and resistance, suggesting that environmental exposure can influence not only community composition but also the degrading capacity of the gut microbiome [7].

However, metagenomic detection of a gene indicates metabolic potential rather than demonstrated activity. A gene may be present but not expressed under the conditions of exposure, and the identification of a predicted metabolic pathway does not, by itself, demonstrate that the corresponding chemical transformation occurs [348]. This limitation has driven the increasing use of functional approaches capable of moving beyond genetic potential towards the measurement of microbial activity.

8.2. Metatranscriptomics and Metaproteomics

Metatranscriptomics provides information on microbial gene expression and can therefore identify pathways that respond to xenobiotic exposure at the transcriptional level. Compared with metagenomics, which primarily describes the genetic potential of a microbial community, metatranscriptomics helps distinguish genes that are present from those that are transcriptionally active under specific experimental conditions [348].

Metaproteomics provides a further level of functional characterisation by measuring proteins expressed by the microbial community [349]. This can be particularly informative for xenobiotic metabolism, since enzymatic activity ultimately depends on the abundance, localisation, and catalytic activity of proteins rather than simply on the presence of their corresponding genes [348].

Together, these approaches can help identify candidate genes, pathways, and enzymes potentially involved in xenobiotic transformation. Neither approach, however, measures enzymatic activity directly, and transcript abundance often correlates poorly with protein abundance in complex microbial communities.

8.3. Metabolomics and Chemical Transformation Mapping

Although metatranscriptomic and metaproteomic approaches can identify candidate microbial functions associated with xenobiotic exposure, changes in transcript or protein abundance do not provide direct evidence that a xenobiotic has been chemically transformed. Demonstrating that a microbial gene or enzyme contributes to xenobiotic transformation requires complementary experimental evidence, typically involving controlled microbial cultures or defined communities together with direct chemical analysis. This is particularly important in complex microbial communities, where a metabolic transformation may involve sequential reactions performed by multiple microorganisms, with an intermediate generated by one species serving as the substrate for a subsequent reaction by another. Consequently, the functional activity of an individual microorganism cannot necessarily be equated with the final metabolic phenotype of the community [350–352].

Metabolomics provides the analytical context required to characterise these chemical consequences. High-resolution mass spectrometry (HRMS) [353], nuclear magnetic

resonance spectroscopy [354], and targeted mass spectrometric approaches [355] enable the detection and characterisation of xenobiotics, microbial transformation products, and endogenous metabolites within complex biological samples.

Untargeted metabolomics is particularly valuable when microbial biotransformation can generate products that are not predicted from conventional host metabolic pathways [356,357]. However, an important challenge is distinguishing genuine xenobiotic-derived products from indirect changes in endogenous metabolism. Stable-isotope tracing provides a powerful solution to this problem. Isotope-labelled xenobiotics can be followed through microbial metabolic pathways, allowing the incorporation of atoms derived from the parent compound into specific transformation products to be demonstrated. When combined with HRMS, isotope tracing can provide strong evidence linking a detected metabolite to the parent xenobiotic and can help reconstruct otherwise unresolved metabolic transformations [358].

Metabolomics can also reveal the sequential nature of microbial xenobiotic metabolism, since transformation products accumulate and are consumed with distinct kinetics. Time-resolved metabolomics can therefore be used to follow the appearance and disappearance of individual chemical species and reconstruct transformation trajectories [359].

Thus, metabolomics provides a critical link between microbial functional activity and chemical mechanisms, enabling the identification, characterisation, and temporal reconstruction of xenobiotic transformation products.

8.4. Gnotobiotic and Humanised Animal Models

Germ-free animals provide a particularly informative experimental model for establishing causal relationships between gut microbiota composition and xenobiotic toxicity, as they can be compared with conventionally colonised animals under controlled exposure conditions [360,361]. Interpretation nevertheless requires caution, as differences observed between germ-free and colonised animals may not be attributable to the absence of microbial metabolism alone.

Gnotobiotic models provide greater mechanistic resolution because animals can be colonised with defined microorganisms or microbial communities [361]. Sequential colonisation experiments can therefore determine whether specific bacterial species, strains, or combinations of microorganisms are sufficient to reproduce a microbiome-dependent phenotype.

Human microbiota-associated or humanised animals extend this approach by introducing human-derived microbial communities into experimental animals [362]. These models can improve translational relevance, particularly when human donor microbiomes display distinct capacities for xenobiotic metabolism.

8.5. Organ-on-a-Chip and Microphysiological Systems

Human intestinal organoids [363] and microphysiological systems [364] provide complementary approaches for investigating the consequences of gut microbiome–xenobiotic interactions. Organoids can reproduce several aspects of intestinal epithelial organisation and cellular diversity, whereas microfluidic systems can introduce controlled flow, mechanical forces, oxygen gradients, and continuous exposure conditions [365,366].

The incorporation of microorganisms into these systems represents an important technological advance. Conventional intestinal cultures are typically maintained under oxygenated conditions that are incompatible with many obligate anaerobic gut microorganisms. Gut-on-chip platforms can overcome some of these limitations by establishing spatially or oxygen-controlled compartments that permit microbial colonisation while maintaining viable intestinal tissue [367]. Since a xenobiotic may be transformed by bacteria into a metabolite that subsequently alters epithelial permeability, inflammatory

signalling, or cellular viability [13], gut-on-chip systems can therefore be used to investigate the biological consequences of microbial transformation directly at the intestinal interface.

Nevertheless, microbiome-enabled organoids and gut-on-chip systems remain technically challenging. Maintaining stable microbial communities, reproducing appropriate oxygen gradients, controlling microbial density, and maintaining long-term epithelial function are major experimental constraints [368]. They also lack the hepatic, immune, and neural compartments required to translate a local transformation into a systemic outcome. As such, these systems have high potential as mechanistic platforms rather than complete representations of the human gastrointestinal tract.

8.6. Single-Cell and Spatial Omics Approaches

Most conventional toxicological analyses of gut-microbiome interactions measure the intestinal response at the tissue level, potentially masking substantial differences between individual cell populations [369]. Single-cell RNA sequencing and related single-cell technologies can overcome this limitation by characterising host responses at cellular resolution [370,371].

In microbiome–xenobiotic research, these approaches can identify whether chemical exposure preferentially affects specific intestinal epithelial populations, immune cells, or other cellular compartments [372]. This may be particularly relevant when the same xenobiotic produces apparently modest changes at the tissue level but strongly affects a specific cellular population.

Spatial transcriptomics provides an additional dimension by retaining information about the anatomical location of molecular signals [373]. This is highly relevant to microbiome research, as microbial-host interactions are spatially organised. Microbial communities and metabolites are not distributed uniformly throughout the intestine, and host responses may differ substantially between the luminal surface, mucus layer, epithelial compartment, and deeper tissue [374–376].

Emerging spatial metabolomics approaches offer the possibility of extending this concept to chemical metabolites [377], allowing microbial transformation products and host metabolic responses to be mapped within tissue. Although these approaches are still developing in the context of xenobiotic toxicology, their combination with microbial sequencing could provide valuable information about where microbiome-mediated chemical transformations occur and which host cells are exposed to their products.

8.7. Computational Modelling and Prediction of Microbiome-Mediated Metabolism

The integration of multi-omics data has led to the development of computational tools capable of predicting microbiome-mediated xenobiotic metabolism [378,379]. Computational approaches are increasingly important for interpreting the large number of microbial genes, chemical structures, metabolites, and metabolic reactions generated by modern microbiome studies. For xenobiotic research, these models can be used to identify candidate metabolic pathways and predict potential transformation reactions [380]. Community-scale metabolic models can additionally account for cross-feeding and metabolic dependencies between microorganisms, which may be important when xenobiotic transformation occurs through sequential reactions involving several species [381].

Machine-learning approaches provide a complementary strategy for predicting relationships between microbial features and xenobiotic transformation. By combining chemical information with microbial enzyme and metabolic data, predictive models can help prioritise candidate organisms, enzymes, and transformation reactions for experimental investigation [378,379].

However, it is important to consider that computational predictions require experimental confirmation, particularly when they are intended to support mechanistic or toxicological conclusions.

9. Therapeutic and Clinical Implications

The recognition of the gut microbiome as an active determinant of xenobiotic metabolism and toxicity raises important translational questions. If microbial activity can modify the fate and toxicity of xenobiotics, deliberate manipulation of the gut microbiome may provide a strategy to improve therapeutic outcomes or reduce xenobiotic-induced toxicity. The therapeutic potential of microbiota-targeted interventions is increasingly recognised, but the field remains at an early stage. Microbiota-targeted interventions have traditionally focused on modifying microbial composition or restoring intestinal homeostasis, whereas their potential to specifically alter xenobiotic metabolism is only beginning to be explored. This distinction is important because changing the microbiome does not necessarily produce a predictable change in xenobiotic metabolism. The relevant target is not simply a healthy microbial community, but the specific microbial functions responsible for the transformation, activation, inactivation, sequestration, or elimination of a particular xenobiotic [26,116,156].

9.1. Microbiome-Targeted Modulation of Xenobiotic Metabolism

Probiotics and prebiotics represent the most established approaches for deliberately modifying the gut microbiome. Probiotics introduce selected live microorganisms, whereas prebiotics are substrates that are selectively utilised by host microorganisms to confer a health benefit. Postbiotics provide a different strategy, using preparations of inanimate microorganisms and/or their components that confer a health benefit on the host [382]. These interventions could theoretically influence xenobiotic toxicity through several mechanisms, including changes in microbial community structure, competition for metabolic substrates, modulation of microbial enzyme activity, alteration of intestinal barrier function, and changes in the production of microbial metabolites that regulate host metabolism and inflammation. However, their potential should not be evaluated simply in terms of their ability to restore a healthy microbiome. Microbial metabolism of xenobiotics is highly function-specific, and taxonomically similar communities may differ substantially in their capacity to transform a given compound. Conversely, taxonomically distant microorganisms may carry functionally similar metabolic activities. Therefore, an intervention that changes microbial abundance does not necessarily produce a predictable change in xenobiotic biotransformation [26,156].

This functional variability has been demonstrated experimentally. Zimmermann et al. [383] showed that human gut bacteria can metabolise a broad range of drugs through microbiome-encoded enzymes, and that the magnitude and pattern of metabolism varied between bacterial species. Similarly, Javdan et al. [209] developed a microbiota-derived metabolism screening framework and demonstrated that drug metabolism by individual-specific microbial communities varies between individuals, identifying previously unrecognised drug-microbiome interactions. More recently, systematic mapping of interactions between approximately 150 dietary xenobiotics and human gut microbial communities demonstrated that microbial metabolism can either decrease or increase the toxicity of xenobiotics and that these responses vary between individuals [350].

This functional perspective also changes how probiotics and prebiotics should be considered in toxicology. Rather than asking whether an intervention increases or decreases a particular bacterial taxon, future studies should determine whether it changes the specific microbial reactions responsible for xenobiotic transformation. For example, an intervention could be beneficial if it decreases the abundance or activity of

microorganisms responsible for bioactivation of a toxic compound, while enhancing pathways that promote detoxification or elimination. Conversely, an apparently beneficial change in microbial composition could be undesirable if it increases the formation of a toxic metabolite. Microbiota modulation should therefore be evaluated according to its effect on xenobiotic fate and biological activity, rather than on microbiota composition alone.

Postbiotics may offer an additional strategy, since they do not require the establishment of viable microorganisms in the intestine [382]. Microbiota-derived molecules and microbial components can directly influence host metabolic, immune, and epithelial pathways, potentially allowing some consequences of dysbiosis to be modified without attempting to reconstruct an entire microbial ecosystem [382,384]. This approach may be particularly attractive when the toxicological consequence of microbiome disruption is mediated indirectly through altered host signalling rather than through direct microbial transformation of the xenobiotic. Nevertheless, the effects of microbial metabolites are often pleiotropic, and the same metabolite may influence several biological pathways. Consequently, postbiotic interventions require careful characterisation of dose, exposure, biological targets, and potential unintended effects [384].

Importantly, direct evidence that probiotics, prebiotics, or postbiotics can reproducibly modify the toxicity of specific xenobiotics in humans remains limited. Much of the current evidence derives from experimental models, and studies frequently assess changes in microbiota composition, inflammation, barrier function, or general host health rather than directly measuring xenobiotic biotransformation and toxicokinetics [385–387]. This limitation is particularly evident for environmental toxicants. For example, experimental studies have explored probiotic, prebiotic, and postbiotic strategies against mycotoxin-associated toxicity, with proposed mechanisms including reduced chemical bioacessibility through binding of the toxin to the microbial cell wall, genoprotection, and attenuation of oxidative or inflammatory responses. However, the available evidence remains heterogeneous and requires confirmation in well-controlled human studies [388]. Thus, microbiome-targeted interventions should currently be regarded as a promising strategy for modifying xenobiotic responses rather than an established approach for mitigating xenobiotic toxicity.

9.2. Incorporating Microbiota Variability into Clinical Studies

The substantial interindividual variability in gut microbial metabolism has important implications for clinical research, as differences in gut microbiota composition and microbiome function may contribute to variability in the safety and efficacy of xenobiotics.

Conventional drug development generally considers host genetics, age, sex, organ function, concomitant medication, and other clinical factors when interpreting variability in drug exposure and response. The gut microbiome is still not routinely incorporated as a determinant of pharmacokinetic or toxicological variability, despite its capacity to chemically modify xenobiotics [26,156]. This omission may be particularly relevant for orally administered compounds with substantial intestinal exposure, low systemic bioavailability, enterohepatic recirculation, or known microbial metabolic pathways.

A more microbiome-informed clinical strategy could therefore include microbiome characterisation alongside conventional pharmacokinetic and pharmacodynamic measurements. Baseline microbial composition, microbial functional capacity, and, where feasible, microbial metabolic activity could be assessed before exposure to the compound under investigation. Longitudinal sampling during treatment could then determine whether the compound itself modifies the microbiome and whether these changes coincide with alterations in drug metabolism, metabolite production, efficacy, or toxicity. Importantly, functional measurements may be more informative than taxonomic

composition alone, since the abundance of a bacterial species does not necessarily predict its functional capacity to transform a given xenobiotic [209,383].

This approach could help explain heterogeneous clinical responses. Instead of treating microbiome variability solely as an uncontrolled source of variance, clinical studies could investigate whether specific microbial genes, enzymes, metabolic pathways, or metabolite profiles predict exposure to active or toxic transformation products. This would move microbiome research from descriptive association towards clinically testable biomarkers of drug disposition and toxicity. Recent reviews have emphasised that interindividual differences in gut microbial metabolism may have consequences for drug efficacy and safety and represent an emerging opportunity for personalised pharmacotherapy [30,156]. There is precedent for this in oncology, where microbiome composition has already been used to stratify patients. The abundance of *A. muciniphila* has been associated with response to immune checkpoint inhibition, and faecal microbiota transplantation has been reported to restore responsiveness in non-responders [389]. The toxicological question posed here is formally similar (whether a microbial feature predicts an individual's internal exposure and response) and the oncological experience suggests both that such stratification is achievable and that it requires functional rather than purely taxonomic measures.

However, incorporating the microbiome into clinical trials presents important practical challenges. The microbiome is dynamic and is influenced by diet, medication use, age, geography, disease status, lifestyle, and other environmental factors. Microbial composition can also vary substantially over time within the same individual [192]. Consequently, a single faecal sample obtained before treatment may not adequately represent the functional state of the microbiome throughout a clinical trial, and faecal samples may not reflect microbial activity at the proximal intestinal sites where many orally administered compounds are absorbed. Standardised sampling procedures, dietary and medication records, longitudinal measurements, and harmonised analytical pipelines will therefore be necessary to distinguish stable microbiome-related determinants from transient changes induced by treatment or other exposures.

An additional challenge is determining what should actually be measured. Taxonomic profiling alone may provide insufficient information for predicting xenobiotic metabolism [209]. Future clinical studies may therefore benefit from combining metagenomics with metatranscriptomics, metaproteomics, metabolomics, and direct measurements of xenobiotic transformation products. Such approaches could identify not only which microorganisms are present, but whether the relevant metabolic functions are present, expressed, and associated with the formation of biologically active metabolites. This functional characterisation may ultimately be more informative for predicting xenobiotic response than microbiota composition alone.

For compounds known or suspected to undergo microbial transformation, microbiome-related endpoints could be incorporated prospectively into study design rather than restricted to retrospective analyses. Ultimately, the clinical significance of the gut microbiome will depend on whether microbial information can improve decisions that matter to patients. The most useful applications are unlikely to come from simply defining an individual's microbiome as healthy or dysbiotic. Instead, the objective should be to determine whether an individual's microbiome has the functional capacity to alter the fate of a particular xenobiotic in a clinically relevant manner. This could create the possibility of combining two complementary strategies: predicting microbiome-dependent xenobiotic metabolism before treatment and, when clinically justified, modifying that metabolism to improve efficacy or reduce toxicity. Although this concept remains largely theoretical, the increasing availability of functional microbiome profiling, ex vivo metabolism assays, and

microbiota-derived metabolic screens makes it increasingly feasible to test in clinical development [30,209,383].

10. Conclusions

Three conclusions of differing evidential weight follow from this review. First, what is established. The gut microbiome is increasingly recognised as an important determinant of xenobiotic disposition and toxicity. Beyond its established roles in host physiology [390], this metabolically active microbial community can directly transform xenobiotics, altering their chemical identity, biological activity, bioavailability, and systemic exposure. These effects may be further amplified by indirect microbiome-mediated mechanisms involving intestinal and BBB integrity, immune regulation, endocrine signalling, and neuronal communication through the gut–brain axis. Together, these interconnected processes provide multiple routes through which the gut microbiome may influence CNS homeostasis and modify susceptibility to xenobiotic-induced neurotoxicity.

Second, what remains plausible but unproven. Current evidence indicates that the toxicological consequences of xenobiotic exposure cannot always be inferred from the properties of the parent compound alone. Microbial metabolism can generate metabolites with reduced, enhanced, or qualitatively distinct biological activities, while interindividual differences in microbiota composition and, more importantly, in microbiome functional capacity may determine which transformations occur and to what extent. Consequently, variability in the gut microbiome represents a potential source of heterogeneity in xenobiotic exposure and neurotoxic responses. The growing evidence from experimental studies, together with the broader relevance of gut microbiome–brain interactions in neurological and neurodegenerative disorders, further supports the need to consider microbial activity when investigating factors that influence CNS vulnerability. This proposition is better supported for pharmaceuticals than for environmental contaminants. For drugs, microbial transformation has been shown to alter efficacy in humans and, in the case of sorivudine, to cause death. For environmental neurotoxicants, the chemistry is demonstrated but the toxicological consequence in humans is not, because the luminal, portal and central concentrations that would establish it have seldom been measured. The proposition that the gut microbiome modifies human neurotoxicity from environmental exposure is, at present, a well-founded hypothesis rather than a finding.

Third, what follows for practice. If microbiome functional capacity determines which transformations occur, then it constitutes a source of interindividual variability in internal dose that current risk assessment does not capture. Regulatory toxicology accommodates variability arising from host genetics, age, sex and organ function, but assumes a constant relationship between external exposure and internal dose with respect to the microbiota. For compounds that undergo substantial microbial transformation, and particularly for orally ingested neurotoxicants with prolonged colonic residence, that assumption may not hold, and the default uncertainty factors applied to interindividual variation may not accommodate it. The practical implication is not that microbiome data should be required in risk assessment today, since the evidence does not support that scenario, but that microbial transformation should be considered when selecting compounds for which internal dose is likely to be poorly predicted by external exposure.

Progress in this field will depend on moving beyond descriptive associations between microbial composition and toxicological outcomes. Advances in metagenomics, functional multi-omics, stable-isotope tracing, gnotobiotic models, microbiome-enabled microphysiological systems, single-cell and spatial approaches, and computational modelling are creating new opportunities to identify the microbial functions responsible for xenobiotic transformation and to establish their consequences for the host. However, integrating these complementary approaches will be essential to connect microbial genes

and enzymes with demonstrated chemical transformations, systemic exposure, and ultimately neurotoxic outcomes.

An important longer-term objective is therefore to incorporate the gut microbiome as a functional component of xenobiotic toxicology. This will require establishing causal and temporal relationships between microbial activity, xenobiotic metabolism, metabolite exposure, and neurotoxicological effects, while accounting for the substantial interindividual and temporal variability in the microbiome. Such knowledge could ultimately support microbiome-informed pharmacokinetic and toxicological models, improve the prediction of individual susceptibility and therapeutic responses, and enable the development of interventions directed at specific microbiome functions rather than microbiota composition alone. Although these applications remain at an early stage, understanding how microbial metabolism intersects with gut–brain communication may provide an important step towards a more comprehensive and mechanistically informed assessment of xenobiotic-induced neurotoxicity.

11. Future Perspectives and Challenges

The increasing evidence that the gut microbiome can modify xenobiotic metabolism and influence the formation, systemic availability, and biological activity of xenobiotic-derived metabolites highlights its potential relevance to neurotoxicity. However, important gaps remain in establishing when microbiome-mediated metabolic changes are sufficient to alter exposure of the nervous system and contribute to neurological injury. Future research should therefore move beyond the demonstrations of individual microbial transformations towards determining how these processes influence neurotoxic outcomes in humans, how they vary between individuals and over time, and whether they can be predicted or therapeutically modulated.

These gaps are not of equal importance, and we set them out in what we judge to be their order of priority. First, and most limiting, is the absence of quantitative exposure data. Luminal, portal and CNS concentrations of microbially derived metabolites are almost never measured, and until they are, the question of whether a demonstrated transformation is toxicologically relevant cannot be answered for any compound. Second are longitudinal human cohorts pairing functional metagenomics, rather than taxonomic profiling alone, with neurological outcome and with measured biomarkers of exposure. Third are gnotobiotic and defined-community models capable of establishing causality for a single named enzyme, since association studies do not support this. Fourth are microphysiological systems coupling a microbial compartment to an intestinal epithelium and a BBB model, which offer the only tractable route to measuring transfer across both barriers in a controlled system. A fifth need, less developed than the others, concerns coverage. The evidence base is dominated by pharmaceuticals, and emerging contaminants such as per- and polyfluoroalkyl substances, microplastic-associated additives and current-use pesticides have scarcely been examined for microbial transformation at all. The subsections that follow address these needs in turn.

11.1. Integrating Microbiome-Mediated Metabolism into Pharmacokinetic and Pharmacodynamic Models

One important direction is the integration of microbial metabolism into pharmacokinetic and pharmacodynamic studies. Conventional models of xenobiotic disposition primarily describe host processes, namely absorption, distribution, metabolism, transport, and excretion, whereas microbial transformations are often considered separately. However, the gut microbiome can influence drug bioavailability, intestinal metabolism, enterohepatic recirculation, and toxicity, and microbial metabolites may differ substantially from the parent compound [391,392]. Consequently, microbial metabolism may

contribute to interindividual differences in systemic exposure and pharmacological or toxicological responses [29].

This consideration may be particularly important for neurotoxicants. Microbial transformation can alter the chemical species available for absorption and systemic distribution, potentially changing the amount and identity of compounds reaching tissues outside the intestine. Some microbial metabolites may also influence neurological responses indirectly through immune, metabolic, endocrine, or gut–brain signalling pathways [392,393]. However, establishing the contribution of these processes to neurotoxicity will require quantitative information on microbial transformation rates, intestinal absorption of the resulting metabolites, systemic concentrations, tissue distribution, including across the BBB, and biological activity.

Future pharmacokinetic models should therefore consider the microbiome as an additional metabolic compartment rather than treating microbial metabolism as an isolated phenomenon. Recent advances in microbiome-aware drug development have already highlighted the potential of incorporating microbial metabolic pathways into pharmacokinetic and pharmacodynamic analyses, and of using microbiome-derived biomarkers to improve the prediction of individual drug responses [394]. Extending these approaches to environmental xenobiotics and neurotoxicants could help determine whether differences in microbial metabolism contribute significantly to variations in neurotoxicity susceptibility. However, before microbiome-derived information can be incorporated into clinical pharmacokinetic or pharmacodynamic decision-making, its predictive value will need to be established in prospective human studies and shown to provide information beyond conventional host-based pharmacokinetic parameters.

11.2. From Microbiota Composition to Functional Prediction of Neurotoxicity

A second major challenge is the prediction of individual susceptibility to neurotoxicity from microbiota characteristics. Although substantial interindividual variation exists in gut microbial composition and metabolic capacity, taxonomic composition alone is unlikely to provide a sufficiently accurate prediction of xenobiotic metabolism and toxicity [209]. Consequently, future studies should prioritise functional measurements, including microbial genes, transcripts, proteins, enzymatic activities, and transformation products, rather than relying exclusively on taxonomic profiles. Integration of metagenomics, metatranscriptomics, metaproteomics, metabolomics, enzyme activity assays, and direct measurements of xenobiotic metabolites could help determine whether relevant metabolic pathways are present, expressed, and functionally active [394]. This approach may be particularly valuable for neurotoxicants because the toxicological consequence is likely to depend not simply on whether a microorganism is present, but on whether microbial activity changes the formation or availability of biologically active compounds.

Thus, the goal should be to identify functional microbial features that can predict the fate of a particular xenobiotic and its potential neurotoxicological consequences. Such biomarkers could eventually contribute to microbiome-informed risk assessment or pharmacological decision-making, although their clinical validity will require prospective testing in well-characterised human populations [7,394]. This translation will also require analytical standardisation and reproducible measurement strategies, while raising ethical considerations if microbiome-derived information is used to classify individuals according to their predicted susceptibility to xenobiotic-induced toxicity.

11.3. Microbiome Editing as a Strategy to Modify Xenobiotic Toxicity

The possibility of deliberately modifying microbial xenobiotic metabolism represents another important area for future research. If specific microbial pathways promote the formation of neurotoxic metabolites, selectively reducing their activity could potentially

decrease toxicity. Conversely, enhancing microbial pathways associated with detoxification or elimination could reduce systemic exposure to harmful compounds. This concept moves beyond the general objective of restoring a healthy microbiota and focuses on modifying specific microbial functions relevant to xenobiotic disposition.

Several technologies are beginning to make targeted microbiome manipulation technically feasible. These include defined microbial consortia [395], engineered probiotics [396], and in situ targeted genetic modification of gut microorganisms [397,398]. For example, Brödel et al. [397] demonstrated in situ base editing of *Escherichia coli* colonising the mouse gut using a phage-derived delivery system, achieving efficient and sustained modification of a targeted bacterial gene following a single administration. Importantly, edited bacteria persisted in the gut for at least 42 days, demonstrating the feasibility of directly modifying bacterial genes within the intestinal environment. More recently, Gelsinger et al. [398] developed a metagenomic editing platform based on clustered regularly interspaced short palindromic repeats (CRISPR)-associated transposases that enabled targeted genetic modification of native gut bacteria in vivo, including *Bacteroides* and segmented filamentous bacteria. This platform enabled the integration of functional genetic payloads into individual bacterial genomes within native microbial communities, extending targeted microbiome engineering beyond experimentally introduced bacterial strains. Although such approaches have not yet been established for controlling xenobiotic-induced neurotoxicity, they provide a potential technological foundation for future microbiome-directed interventions.

Nevertheless, the therapeutic manipulation of the gut microbiome raises important safety and regulatory challenges, as microbiome editing may modify the host evolutionary potential [399]. In the context of gut–brain axis-mediated effects on xenobiotic-induced neurotoxicity, microbiome manipulation should be evaluated according to its effect on xenobiotic fate and toxicity rather than simply according to changes in microbial composition. Modifying one microbial pathway may affect other metabolic reactions, including those involving therapeutic drugs, dietary compounds, and endogenous compounds, as well as microbial interactions or host signalling pathways [400,401]. Consequently, future studies will need to establish both the intended metabolic effect and potential off-target consequences. This will be essential to establish a causal link between microbiome manipulation and its consequences for the neurotoxicity of xenobiotics mediated by the gut–brain axis.

11.4. Establishing Causality and Temporal Relationships Between the Gut Microbiome and Neurotoxicity

Another major challenge is establishing whether microbiome-mediated metabolic changes are a cause, consequence, or modifier of xenobiotic-induced neurotoxicity [402]. The bidirectional relationship between the gut microbiome and xenobiotic exposure makes temporal relationships particularly difficult to establish, since the same exposure that is transformed by the microbiota may simultaneously alter its composition and function. Thus, longitudinal studies are needed to characterise microbiome–xenobiotic interactions before, during, and after exposure. Recent human evidence has shown that environmental pollutant exposure is associated with changes in both gut microbial composition and xenobiotic-related microbial functions [7], highlighting the need to determine how these changes evolve over time. This is highly relevant, as changes in the microbiome may persist beyond the period of xenobiotic exposure [403]. However, longitudinal human studies will require careful consideration of informed consent, data interpretation, and long-term monitoring [404,405]. These considerations will become particularly important when microbiome-targeted interventions are combined with repeated exposure assessment.

Additionally, repeated measurements of microbial composition and function should ideally be combined with measurements of parent xenobiotics, microbial transformation products, systemic biomarkers, and neurotoxicological outcomes. Such designs could distinguish stable characteristics of the microbiome from transient microbial responses induced by exposure. Complementary experimental approaches, including gnotobiotic models, defined microbial communities, targeted genetic manipulation of gut bacteria, stable-isotope tracing, and human-derived gut–brain models, could further help establish causality by determining whether disruption of a specific microbial pathway alters the formation of neuroactive or neurotoxic metabolites and, ultimately, their contribution to xenobiotic-induced neurotoxicity.

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Abbreviations

The following abbreviations are used in this manuscript:

5-ASA	5-Aminosalicylate
5-FU	5-Fluorouracil
5-HT	5-Hydroxytryptamine (serotonin)
5-HTP	5-Hydroxytryptophan
Aβ	Amyloid-β peptide
Acetyl-CoA	Acetyl-coenzyme A
ACTH	Adrenocorticotropic hormone
AD	Alzheimer's disease
AhR	Aryl hydrocarbon receptor
ANS	Autonomic nervous system
APP	Amyloid precursor protein
ATP	Adenosine-5′-triphosphate
BBB	Blood–brain barrier
CCK	Cholecystokinin
CNS	Central nervous system
CYP450	Cytochrome P450 metabolic system

DPD	Dihydropyrimidine dehydrogenase
ENS	Enteric nervous system
FFAR	Free fatty acid receptor
GABA	γ-aminobutyric acid
GLP-1	Glucagon-like peptide-1
GPCR	G protein-coupled receptor
GRE	Glycyl radical enzyme
HPA	Hypothalamic–pituitary–adrenal
HRMS	High-resolution mass spectrometry
IBD	Inflammatory bowel disease
IgA	Immunoglobulin A
LPS	Lipopolysaccharide
MDD	Major depressive disorder
MS	Multiple sclerosis
MUFA	Monounsaturated fatty acid
NAS	Non-caloric artificial sweeteners
NSAIDs	Non-steroidal anti-inflammatory drugs
PD	Parkinson's disease
P-gp	P-glycoprotein
PNS	Peripheral nervous system
PS1	Presenilin 1
PUFA	Polyunsaturated fatty acid
PXR	Pregnane X receptor
RNA	Ribonucleic acid
SAM	S-adenosylmethionine
SCFA	Short-chain fatty acid
SN-38	7-Ethyl-10-hydroxycamptothecin
SN-38G	7-Ethyl-10-hydroxycamptothecin -glucuronide
SULT	Sulphotransferase
TLR	Toll-like receptor
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
TNT	2,4,6-Trinitrotoluene
TPH	Tryptophan hydroxylase
UGT	Uridine diphosphate glucuronosyltransferase

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