

Concept Article

AOPs to Connect Food Additives' Effects on Gut Microbiota to Health Outcomes

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Received November 27, 2024;

Accepted December 2, 2024;

Epub January 14, 2025;

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ALTEX 42(2), 257-262.
doi:10.14573/altex.2411271

Abstract

Gut microbiota play a central role in human health, notably through the production of metabolites, including short-chain fatty acids, secondary bile acids, vitamins or neurotransmitters. Beyond contributing to gut health, these microbial metabolites significantly impact multiple organ systems by activating key signaling pathways along the gut-organ axes, including the gut-liver, gut-brain, and gut-bone axes. Chemicals ingested through food such as food additives, extensively used to enhance the texture, preservation and appearance of foods, may interact with our gut microbiota, altering metabolite production, and this can have consequences for our health. However, gut microbial metabolism is currently overlooked in toxicology. While efforts are underway to develop standardized human-based new approach methodologies to assess compound-microbiome interactions, anchoring those assays within the adverse outcome pathway (AOP) framework would offer a structured way to connect changes in gut microbial metabolism to adverse health outcomes. Using human-based models enhances the relevance of the results while supporting the reduction of animal-based testing in toxicology research.

Plain language summary

The microorganisms in our gut release molecules that influence our body functions and keep us healthy. Food additives, which are used to enhance the texture, preservation and appearance of foods, may disrupt the microbial balance, potentially leading to detrimental health effects. However, the potential impact of food additives on gut microorganisms is not yet considered in their safety assessments. Developing human-based tests to study these interactions could inform the safety assessment of food additives. The adverse outcome pathway (AOP) framework allows compiling and structuring relevant information and identifying knowledge gaps. Using human-based tests rather than animal tests yields more relevant results and reduces the reliance on animal testing in toxicology research.

1 Potential toxicity of food additives on human gut microbiota

Each year, the Society of Toxicology (SOT) and the Federation of European Toxicologists & European Societies of Toxicology (EUROTOX) congresses feature a debate in which leading toxicologists advocate opposing sides of a topic of significant toxicological importance. In 2024, the debate addressed the question: “*Can the microbiome mediate the toxicity of environmental chemicals?*” (Beekmann and Tal, 2024). The debaters discussed how the community of microorganisms hosted in our gut might influence the toxic effects of chemicals, but also briefly touched on the reciprocal question – How do chemicals impact the microbiome? In this regard, one debater argued that several examples of chemicals and lifestyle factors that alter the composition of the intestinal microbiota have been well documented, such as alcohol,

antibiotics or diet. The opposing viewpoint emphasized the current lack of evidence to establish causality and stressed that microbiome composition is not necessarily a predictor of functional changes nor consequences on host health. Although both audiences voted “yes” on the lead question, it was unanimously acknowledged that our understanding of and ability to prove causation of a chemical-induced altered microbiome and its adverse effects on host health remains limited.

Of particular concern is the alarming knowledge gap when it comes to food chemicals. While humans ingest food additives every day, their impact on our gut microbiota and the associated consequences for health are still unclear. Food additives are substances intentionally added to food to preserve it or to enhance its texture, flavor or appearance (Whelan et al., 2024). Preservatives such as nitrates/nitrites and sulfites, commonly used in processed meats, baked goods, and dried fruits, extend shelf life by prevent-



ing spoilage by bacteria. To mix ingredients that do not merge, such as oil and water, emulsifiers like polysorbate-80 and carboxymethylcellulose are used in processed foods, e.g., margarine or mayonnaise. Artificial sweeteners such as aspartame, sucralose or saccharin, found in diet sodas and sugar-free foods, provide sweetness without the calories of sugar. Flavor enhancers, like monosodium glutamate and disodium inosinate, are commonly found in savory snacks and soups to improve their taste or aroma. Titanium dioxide nanoparticles (E171, banned from the EU market since 2020), tartrazine or beta-carotene are used as color additives in candies and beverages. Thickeners and stabilizers, such as silica nanoparticles, xanthan gum and gelatine, commonly found in sauces, are used to improve food texture. Antioxidants are used to prevent oxidation, e.g., in processed meats.

While food additives offer benefits, they also raise concerns about potential health effects, especially regarding our gut microbiota. Numerous rodent studies have provided insights into their potential impact on gut microbiota mediated via diverse mechanisms. Notably, several recent studies demonstrated that food emulsifiers modify the composition of the gut microbiota (Whelan et al., 2024). Such changes have been linked to low-grade inflammation, metabolic syndrome, and obesity (Chassaing et al., 2015). Regarding artificial sweeteners, aspartame consumption in mice and rats was shown to result in gut dysbiosis (Whelan et al., 2024). Similarly, studies have indicated that food preservatives suppress the growth of beneficial bacteria, altering the overall microbial balance and leading to gut inflammation (Li et al., 2022). While these findings point towards a need to consider the impact of food additives on gut microbiota, translating results from rodents to humans is hampered by differences in microbiota metabolism between species.

Nevertheless, some human-based studies have highlighted similar concerns. Results on human gut microbiota maintained *ex vivo* suggested that numerous commonly used emulsifiers can directly alter the microbiota in a manner expected to promote intestinal inflammation (Naimi et al., 2021). Saccharin and aspartame have been shown to induce dysbiosis in human gut (Gerasimidis et al., 2020; Lehman et al., 2023). But, both in rodents and humans, not all studies report microbiome changes, underlining the varying effects of artificial sweeteners on the gut microbiome and demonstrating the difficulty of interpreting and comparing studies that use different methodologies.

In addition to food additives, humans are exposed to chemicals that are not intentionally added to the food, such as pesticides or silver nanoparticles used for their antibacterial properties on food contact materials. Pesticides, including herbicides, insecticides and fungicides, are widely used in agriculture to protect crops but can inadvertently harm non-target organisms, including the human gut microbiota. Glyphosate, one of the most used herbicides, acts by inhibiting the shikimate pathway, a metabolic pathway vital to produce certain amino acids in bacteria but absent in humans. However, many beneficial gut bacteria rely on this pathway, therefore exposure to glyphosate potentially affects them and indirectly the host. Accordingly, in mice, low glyphosate exposure was shown to alter microbial composition (Lehman et al., 2023).

Despite growing concerns, a comprehensive understanding of the functional changes to gut microbiota caused by food addi-

tive ingestion and their health significance remains largely lacking. Notably, this is because most studies to date investigate only changes in microbial species. While taxonomic profiling is important, it cannot provide insights into functional changes, given that microbiome function can be altered in the absence of changes in the composition of species and *vice versa*.

Moreover, the gut microbiome influences various organ systems beyond the gut, through release of metabolites and their influence on diverse signaling pathways along the well-described gut-organ axes. Integrating gut-organ axes into toxicology is necessary to comprehensively understand how food chemicals might affect health. Significant challenges include understanding the systemic effects caused by microbial metabolites, the complexity of microbiome-host interactions, the high variability of individual microbiomes, and the limited availability of models that accurately replicate compound-microbiota interactions.

To date, studies have mainly used rodents with natural microbiota (conventional), germ-free rodents (without microbiota), and germ-free rodents colonized with human microbiota (Jimonet et al., 2024). These models have given some insight into the possible role of microbiota in mediating food chemical-induced health impacts. However, they are limited by the lack of standardized protocols and the open question of how well rodents predict the human situation. Next to these scientific concerns, the 3R principle (replace, reduce, refine) encourages the development of alternative approaches to reduce the use and suffering of animals in scientific research.

The ideal strategy would be to directly investigate these interactions inside the human body. However, clinical studies are hampered by ethical, regulatory, and budgetary constraints and are often limited to endpoint measurements, where it is more difficult or impossible to capture the mechanisms involved. Hence, elucidating chemical-induced alterations in microbiome functions and their effects on human health requires human-relevant assays that reflect human host signaling pathways and provide insight into the relevant mechanisms. Different interdisciplinary efforts are developing tools and models that are human-based, including computational modelling of microbial communities, *in vitro* incubation of human-derived (pooled) fecal microbiota samples (Jimonet et al., 2024) or gut models, including simple static batch culture systems or complex dynamic models that maintain functional microbiota up to several weeks. While these methodologies are still under development, with diverse consortia working on developing standardized protocols (such as the COST action INFOGUT) to further increase their relevance and work towards potential regulatory implementation, they should also be anchored within a mechanistic framework to provide a robust understanding of the pathways that lead to adverse outcomes.

2 The adverse outcome pathway (AOP) framework can position microbiota in toxicology

In this context, the mechanistic-based approach of the adverse outcome pathway (AOP) framework could be highly useful. An AOP represents a sequence of causally linked biological key events (KE) that begins with the initial interaction of a stressor with a host mol-

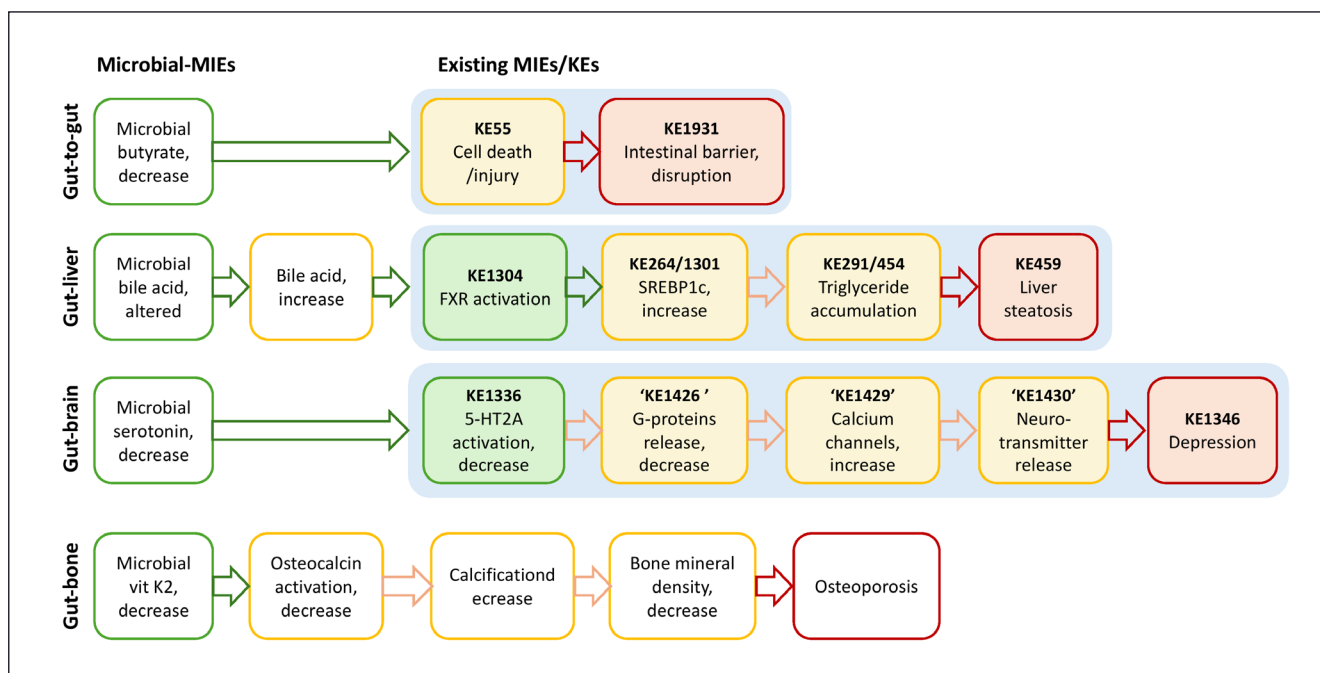


Fig. 1: Four proposed adverse outcome pathways (AOPs) linking new microbial-based molecular initiating events (MIEs) to existing or new key events (KEs) in the gut, liver, brain or bone, leading to an adverse outcome at the organism (human) level

ecule, termed the molecular initiating event (MIE), and ends with an adverse outcome (AO) (Villeneuve et al., 2014). Within this framework, a KE describes a measurable and essential change in a biological system that can be quantified in experimental or clinical settings. Hence, the strength of an AOP lies in its ability to leverage data from molecular and cell-based methods or mathematical models to predict adverse outcomes. By aligning *in vitro/ ex vivo/ in silico* microbial assays to microbial MIE, it could be possible to gain deeper insights into microbiota-driven functional changes and their implications for human health. Some relevant KEs are already well-described in the AOP framework, which is inherently modular, fostering reuse of existing components (Villeneuve et al., 2014). This modularity makes AOPs particularly useful for assessing both local intestinal effects and linking changes in microbial metabolites to broader impacts on other organs.

But, despite their growing recognition as key components in next-generation risk assessment, AOPs have not yet been applied to evaluate toxicity stemming from gut dysbiosis nor the resulting damage to the host. Recently, in the COVID-19 context, the gut microbiota was integrated for the first time within AOPs, either as a factor modulating the outcome (Clerbaux, et al., 2022a) or as an adverse outcome triggered by SARS-CoV-2 as a stressor (Clerbaux, et al., 2022b). Taking this a step further, the conventional applicability of the framework could be extended to link alterations of specific gut microbial metabolites production, as initiating events, to adverse outcomes in the host.

The development of an AOP is a dynamic process building from hypothesized KEs towards refinement of KEs and key event re-

lationships (KERs) by assembling evidence found in literature or newly produced (Vinken, 2018). Thus, an AOP can be instrumental for systematically organizing mechanistic knowledge gained from a prototypical stressor known to induce a biological response, but also to build new mechanistic hypotheses and gather existing or newly produced evidence to support the occurrence of this pathway upon interaction with a stressor of interest. AOP development guidance has been established by the OECD, and developed AOPs describing various KEs and diverse adverse outcomes are stored in the publicly accessible repository AOP-Wiki¹. There is no explicit quality assurance for AOPs in the AOP-Wiki, except when they have undergone the OECD's endorsement process, making those AOPs compliant for potential regulatory use.

Below, four putative AOPs are proposed to illustrate the opportunities of reusing knowledge of adversity (Clerbaux et al., 2024a) to further elucidate the potential toxicity of food additives on microbiota along selected gut-organ axes.

3 Gut-to-gut: Short-chain fatty acids (SCFA) and gut barrier integrity

Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, are key metabolites produced by gut bacteria through the fermentation of dietary fibers. SCFA production is predominantly dependent on the presence of fiber-fermenting bacteria such as *Bifidobacteria* and *Lactobacillus*, which are part of the beneficial microbial community in the gut (Blaak et al., 2020).

¹ <https://aopkb.oecd.org/>



These metabolites play significant roles in maintaining gut health, regulating immune function, and modulating energy metabolism. SCFAs, particularly butyrate, contribute to maintaining the intestinal barrier and promoting the integrity of epithelial cells by serving as the primary energy source for colonocytes and by regulating tight junction proteins (Blaak et al., 2020). As outlined in the AOP-Wiki (KE1930), the intestinal barrier is a multi-layered system with the epithelial layer acting as a protective barrier that separates the internal blood from the luminal contents (Clerbaux, et al., 2022c).

Damage or death of enterocytes leads to increased intestinal permeability, also called “leaky gut”, enabling translocation of bacteria, bacterial products (such as lipopolysaccharides), and undigested nutrients from the intestinal lumen into the systemic circulation. Hence, individuals with a leaky gut might be more vulnerable to chemical toxicity, as dietary pesticides or food chemicals can more easily enter the systemic blood and reach the organs. Moreover, a leaky gut has been associated with several diseases, including diarrhea, inflammatory bowel disease, celiac disease, and type 1 diabetes (Di Vincenzo et al., 2024). This prompted us to propose considering leaky gut as an adverse outcome (Stanco et al., 2024).

Hence, by building on existing KEs while introducing a new microbial-based MIE, an AOP could be designed that links decreased microbial butyrate production, acting as a MIE, to intestinal barrier disruption via cellular damage (Fig. 1).

4 Gut-liver axis: Bile acids and fatty liver diseases

The gut-liver axis highlights the interaction between the gut (microbiota) and the liver via the portal vein, which has implications for lipid, glucose or energy regulation, notably via the bile acids. Bile acids are important signaling molecules that finetune the communication between the gut and the liver (Gillard and Leclercq, 2023). In the liver, primary bile acids such as cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized and conjugated with glycine or taurine before being secreted into the intestine. In the colon, microbiota transform primary bile acids into secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid (LCA) through deconjugation and dehydroxylation processes. These microbial transformations are crucial for regulating their signaling functions in various tissues. Beyond their important role as detergents for lipid digestion, bile acids act as ligands for several host receptors (Gillard and Leclercq, 2023), including the nuclear receptor farnesoid X receptor (FXR). FXR, primarily expressed in the liver and intestine, regulates bile acid synthesis, lipid metabolism, and glucose homeostasis. Activation of FXR by bile acids (particularly primary bile acids, e.g., CDCA) suppresses bile acid synthesis via feedback inhibition but also regulates lipid metabolism.

Excessive activation of FXR – due to an increased concentration of bile acids that can be caused by impaired microbial bile acid metabolism – promotes the accumulation of lipids in the liver, leading to hepatic steatosis (fatty liver). This pathway has been

largely captured within AOPs (Mellor et al., 2016; van der Voet et al., 2020). An *in vitro* test battery to assess alterations in this AOP leading to liver steatosis from FXR activation to changes in gene expression and triglyceride accumulation in hepatocytes has been developed for assessing the steatotic potential of chemicals (Karaca et al., 2023).

Therefore, alterations of microbial bile acid metabolism could be considered as a MIE that increases bile acid concentrations, subsequently triggering FXR activation and downstream KEs leading to liver steatosis (Clerbaux et al., 2024b) (Fig. 1).

5 Gut-brain axis: Serotonin and mood disorders

Another important axis relates to the bidirectional communication between the gut and the brain. Gut bacteria also produce neurotransmitters like serotonin (5-hydroxytryptamine or 5-HT), which plays important roles in bowel motility (Kendig and Grider, 2015) and cardiovascular function (Côté et al., 2004). But serotonin’s most prominent role is in the CNS, where it regulates mood, anxiety, and depression. Serotonin binds to various receptors (5-HT receptors), leading to the release of other neurotransmitters, like dopamine and norepinephrine, which are essential for emotional regulation. Selective serotonin reuptake inhibitors, which increase serotonin availability in the synaptic cleft, are used to treat anxiety and depression. These pathways have been captured within AOPs (AOP235, AOP236, AOP224). AOP236 describes the effect of 5-HT_{2A} receptor agonism in the brain, leading to antidepressant activity via calcium channel inhibition, preventing the flow of calcium ions into neurons, which prevents neurotransmitter vesicle release between neurons.

Considering, on the contrary, depression as the adverse outcome, one could propose an AOP linking reduced microbial serotonin production as MIE that decreases 5-HT receptor activation, subsequently triggering a calcium-mediated increase of neurotransmitter release, leading to depression (Fig. 1).

6 Gut-bone axis: Vitamin K2 and osteoporosis

Gut microbiota also synthesize several essential vitamins, including vitamin K, specifically K₂, and B vitamins. Besides its well-established role in blood coagulation, vitamin K₂ also has been proposed to be involved in bone health. Some studies proposed that vitamin K₂ activates osteocalcin, a protein critical for bone mineralization.

Reduced activation of osteocalcin leads to weakened bones and osteoporosis (Ma et al., 2022). More studies are needed to confirm this pathway, and caution is warranted in interpreting these findings for clinical practice, but this knowledge may inform strategies to mitigate adverse effects of food chemicals on bone health. Bone-related adverse outcomes are absent from the AOP-Wiki, except for bone loss, commonly seen in osteoporosis and widely reported in astronauts, proposed to be triggered by radiation in AOP482 (Sandhu et al., 2024).

A hypothetical AOP could link decreased vitamin K2 microbial production as the MIE, reducing the activation of osteocalcin, decreasing calcification and bone mineralization, and leading to osteoporosis (Fig. 1).

7 Conclusion and perspectives

The alteration of gut microbial metabolites production by food additives might have implications on various organ systems through perturbation of key physiological pathways. Understanding these pathways is essential for developing test strategies to mitigate potential toxicity to gut microbiota and respective consequences for human health.

The AOP framework provides a mechanistic approach to link microbial metabolite alterations as MIEs to adverse outcomes in the gut and other organs. To illustrate this, four AOPs are proposed here: decreased microbial butyrate production leading to intestinal barrier disruption via cellular damage; altered microbial bile acid metabolism increasing bile acid concentrations and triggering FXR activation resulting in liver steatosis; reduced microbial serotonin production decreasing 5-HT receptor activation leading to calcium-mediated neurotransmitter release and depression; and decreased vitamin K2 production impairing osteocalcin activation, decreasing calcification and bone mineralization leading to osteoporosis. These proposed AOPs illustrate that the framework can be instrumental to predict adverse outcomes triggered by food chemicals on the gut but also on other organs. The gut-liver and gut-brain axes are well documented, while the gut-bone axis is emerging. Accordingly, the respective AOPs were built on well-described KEs or, alternatively, were used to serve as hypotheses for a pathway that requires further study. This AOP-aligned approach might also be used for other axes, such as, for example, the gut-lung axis.

Certainly, this approach faces challenges linked to the microbiota field, such as the high variability in individual microbiomes, the complexity of microbiome-host interactions, and the limited availability of standardized methods. Moreover, while there has been an unquestionable dynamism in the microbiota field over the last decades, when it comes to microbial metabolism, we have only uncovered the tip of the iceberg, and there remains a vast hidden world yet to be explored. This incomplete understanding of microbial metabolites-induced systemic effects presents a challenge, as the AOP framework is principally knowledge-driven. Additionally, it is challenging to deal with effect duration (Leist et al., 2017) to capture low-dose ingestion of food chemicals and the resilience of the microbiota ecosystem within the linear unidirectional structure of the AOPs. As mentioned, standardized assays are not yet available for microbial MIEs, though the field is advancing rapidly. For the downstream KEs reused from existing AOPs, methodologies might already exist for some. The challenge will lie in integrating microbial metabolites with host cells in a way that faithfully recapitulates the *in vivo* situation.

In conclusion, while challenging, the AOP framework might offer a valuable opportunity to comprehensively assess the potential

toxicity of food additives on human gut microbiota and its downstream effects on different organs. By providing a mechanistic approach to link microbial metabolite changes to adverse outcomes via KEs linked to appropriate new approach human-based methodologies, AOPs could position gut microbial metabolism as a relevant endpoint in toxicology.

References

- Beekmann, K. and Tal, T. (2024). Can the microbiome mediate the toxicity of environmental chemicals? *Toxicol Lett* 399, S2-S3. doi:10.1016/j.toxlet.2024.07.010
- Blaak, E. E., Canfora, E. E., Theis, S. et al. (2020). Short chain fatty acids in human gut and metabolic health. *Benef Microbes* 11, 411-455. doi:10.3920/bm2020.0057
- Chassaing, B., Koren, O., Goodrich, J. K. et al. (2015). Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* 519, 92-96. doi:10.1038/nature14232
- Clerbaux, L. A., Mayasich, S. A., Muñoz, A. et al. (2022a). Gut as an alternative entry route for SARS-CoV-2: Current evidence and uncertainties of productive enteric infection in COVID-19. *J Clin Med* 11, 5691. doi:10.3390/jcm11195691
- Clerbaux, L. A., Filipovska, J., Muñoz, A. et al. (2022b). Mechanisms leading to gut dysbiosis in COVID-19: Current evidence and uncertainties based on adverse outcome pathways. *J Clin Med* 11, 5400. doi:10.3390/jcm11185400
- Clerbaux, L. A., Albertini, M. C., Amigó, N. et al. (2022c). Factors modulating COVID-19: A mechanistic understanding based on the adverse outcome pathway framework. *J Clin Med* 11, 4464. doi:10.3390/jcm11154464
- Clerbaux, L. A., Filipovska, J., Nymark, P. et al. (2024a). Beyond chemicals: Opportunities and challenges of integrating non-chemical stressors in adverse outcome pathways. *ALTEX* 41, 233-247. doi:10.14573/altex.2307061
- Clerbaux, L. A., Stanco, D., Proot, V. et al. (2024b). Adverse outcome pathways to support assessment of food nanoparticles toxicity on gut microbiota and intestinal barrier integrity. *Toxicol Lett* 399, S128. doi:10.1016/j.toxlet.2024.07.329
- Côté, F., Fligny, C., Fromes, Y. et al. (2004). Recent advances in understanding serotonin regulation of cardiovascular function. *Trends Mol Med* 10, 232-238. doi:10.1016/j.molmed.2004.03.007
- Di Vincenzo, F., Del Gaudio, A., Petito, V. et al. (2024). Gut microbiota, intestinal permeability, and systemic inflammation: A narrative review. *Intern Emerg Med* 19, 275-293. doi:10.1007/s11739-023-03374-w
- Gerasimidis, K., Bryden, K., Chen, X. et al. (2020). The impact of food additives, artificial sweeteners and domestic hygiene products on the human gut microbiome and its fibre fermentation capacity. *Eur J Nutr* 59, 3213-3230. doi:10.1007/s00394-019-02161-8
- Gillard, J. and Leclercq, I. A. (2023). Biological tuners to reshape the bile acid pool for therapeutic purposes in non-alcoholic fatty liver disease. *Clin Sci* 137, 65-85. doi:10.1042/cs20220697



- Jimonet, P., Druart, C., Blanquet-Diot, S. et al. (2024). Gut microbiome integration in drug discovery and development of small molecules. *Drug Metab Dispos* 52, 274-287. doi:10.1124/dmd.123.001605
- Karaca, M., Fritsche, K., Lichtenstein, D. et al. (2023). Adverse outcome pathway-based analysis of liver steatosis in vitro using human liver cell lines. *STAR Protoc* 4, 102500. doi:10.1016/j.xpro.2023.102500
- Kendig, D. M. and Grider, J. R. (2015). Serotonin and colonic motility. *Neurogastroenterol Motil* 27, 899-905. doi:10.1111/nmo.12617
- Lehman, P. C., Cady, N., Ghimire, S. et al. (2023). Low-dose glyphosate exposure alters gut microbiota composition and modulates gut homeostasis. *Environ Toxicol Pharmacol* 100, 104149. doi:10.1016/j.etap.2023.104149
- Leist, M., Ghallab, A., Graepel, R. et al. (2017). Adverse outcome pathways: Opportunities, limitations and open questions. *Arch Toxicol* 91, 3477-3505. doi:10.1007/s00204-017-2045-3
- Li, P., Li, M., Wu, T. et al. (2022). Systematic evaluation of antimicrobial food preservatives on glucose metabolism and gut microbiota in healthy mice. *NPJ Sci Food* 6, 42. doi:10.1038/s41538-022-00158-y
- Ma, M. L., Ma, Z. J., He, Y. L. et al. (2022). Efficacy of vitamin K2 in the prevention and treatment of postmenopausal osteoporosis: A systematic review and meta-analysis of randomized controlled trials. *Front Public Health* 10, 979649. doi:10.3389/fpubh.2022.979649
- Mellor, C. L., Steinmetz, F. P. and Cronin, M. T. D. (2016). The identification of nuclear receptors associated with hepatic steatosis to develop and extend adverse outcome pathways. *Crit Rev Toxicol* 46, 138-152. doi:10.3109/10408444.2015.1089471
- Naimi, S., Viennois, E., Gewirtz, A. T. et al. (2021). Direct impact of commonly used dietary emulsifiers on human gut microbiota. *Microbiome* 9, 66. doi:10.1186/s40168-020-00996-6
- Sandhu, S., Keyworth, M., Karimi-Jashni, S. et al. (2024). AOP report: Development of an adverse outcome pathway for deposition of energy leading to bone loss. *Environ Mol Mutagen* 65, Suppl 3, 85-111. doi:10.1002/em.22631
- Stanco, D., Lipsa, D., Bogni, A. et al. (2024). An adverse outcome pathway for food nanomaterial-induced intestinal barrier disruption. *Front Toxicol* 6, 1474397. doi:10.3389/ftox.2024.1474397
- van der Voet, H., Kruisselbrink, J. W., de Boer, W. J. et al. (2020). The MCRA toolbox of models and data to support chemical mixture risk assessment. *Food Chem Toxicol* 138, 111185. doi:10.1016/j.fct.2020.111185
- Villeneuve, D. L., Crump, D., Garcia-Reyero, N. et al. (2014). Adverse outcome pathway (AOP) development I: Strategies and principles. *Toxicol Sci* 142, 312-320. doi:10.1093/toxsci/kfu199
- Vinken M. (2018). Taking adverse outcome pathways to the next level. *Toxicol In Vitro* 50, A1-A2. doi:10.1016/j.tiv.2018.03.017
- Whelan, K., Bancil, A. S., Lindsay, J. O. et al. (2024). Ultra-processed foods and food additives in gut health and disease. *Nat Rev Gastroenterol Hepatol* 21, 406-427. doi:10.1038/s41575-024-00893-5

Conflict of interest

The author declares she has no conflict of interest.

Data availability

No datasets were generated or analyzed for this manuscript.

Acknowledgements

The author acknowledges funding from the Brains for Brussels program supported by Innoviris – Brussels Capital Region (2023-BFB-1).